Chemical-ecological interaction among *Epiphyas postvittana* (Lepidoptera: Tortricidae), *Botrytis cinerea* (Helotiales: Sclerotiniaceae), and *Vitis vinifera* (Vitales: Vitaceae)

Syed Zulfiqar Mehdi Rizvi
M.Sc. Biotechnology
M.Sc. Plant Physiology
B.Sc. (Hons) Botany

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Charles Sturt University

Faculty of Science
School of Agricultural & Wine Sciences
March 2016
To my family members
CHAPTER 1  EPIPHYAS POSTVITTANA–VITIS VINIFERA–BOTRYTIS CINEREA: THREE-WAY INTERACTING SYSTEM

1. GENERAL INTRODUCTION .......................................................... 3
   1.1. Insect–fungus–plant interaction: three-way interactions .................... 3
   1.1.1. Insect–plant interactions ................................................ 3
   1.1.2. Fungal association in insect–plant interactions .......................... 4
   1.1.3. Fungal infection influences the life-history performance of plant-feeding insects ...... 6
   1.1.4. Fungal infection alters the oviposition preference of plant-feeding insects .......... 8
   1.2. Theories that explain such interactions .................................... 9
   1.2.1. Preference—performance hypothesis ................................... 9
   1.2.2. Selfish-motherhood hypothesis ....................................... 10
   1.2.3. Transmissive-mother hypothesis ...................................... 11
   1.3. Participants in this study .................................................. 11
       1.3.1. Vitis vinifera ......................................................... 12
       1.3.2. Epiphyas postvittana ................................................ 12
           1.3.2.1. Economic impact ............................................. 16
           1.3.2.2. Phenology ..................................................... 17
           1.3.2.5. Host range ..................................................... 19
           1.3.2.6. Host selection ................................................. 20
           1.3.2.7. Host susceptibility ............................................ 21
           1.3.2.8. Management .................................................. 22
       1.3.3. Botrytis cinerea ..................................................... 23
           1.3.3.1. Biology of Botrytis cinerea ................................. 23
           1.3.3.2. Geographical incidence and host range ........................ 24
           1.3.3.3. Interaction with grapes ..................................... 25
   2. THE PURPOSE OF THIS STUDY AND RESEARCH QUESTIONS .................. 29
   3. REFERENCES ...................................................................... 30

CHAPTER 2  VARIATIONS IN THE CHEMISTRY IN THE LEAVES OF FIVE VARIETIES OF VITIS VINIFERA AND THEIR IMPLICATIONS IN THE PERFORMANCE AND DEVELOPMENT OF EPIPHYAS POSTVITTANA*

1. INTRODUCTION ...................................................................... 43
2. MATERIALS AND METHODS .................................................. 45
   2.1. Insect culture ................................................................. 45
   2.2. Lyophilization of leaf tissues of different varieties of V. vinifera .............. 46
   2.3. Leaf chemistry ............................................................... 47
   2.4. Synthetic diets for the larvae .............................................. 47
   2.5. Life history performance of E. postvittana .................................. 48
       2.5.1. Growth and development ............................................ 48
       2.5.2. Adult performance .................................................. 49
       2.5.3. Female–fitness index ............................................... 49
       2.5.4. Discriminant analysis .............................................. 51
   2.6. Statistical analysis .......................................................... 51
3. RESULTS .............................................................................. 51
   3.1. Leaf chemistry ............................................................... 51
   3.2. Life history performance of E. postvittana .................................. 52
       3.2.1. Growth and development ............................................ 52
       3.2.2. Adult performance .................................................. 52
       3.2.3. Female–fitness index ............................................... 53
       3.2.4. Discriminant analysis .............................................. 53
CHAPTER 3  INFLUENCE OF \textit{BOTRYTIS CINerea} INFECTED LEAVES OF \textit{VITIS VINIFERA} ON THE PREFERENCE OF \textit{EPIPHYas POSTVITTANA}\textsuperscript{*}

1. INTRODUCTION ...................................................................................................................... 73
2. MATERIAL AND METHODS .............................................................................................. 75
   2.1. Insect culture .................................................................................................................. 75
   2.2. Fungus culture and preparation of spore suspension ......................................................... 75
   2.3. Plant culture .................................................................................................................... 76
   2.4. Level of infection of \textit{V. vinifera} leaves with \textit{B. cinerea} .............................................. 77
   2.5. Bioassay of oviposition behaviour ................................................................................. 77
      2.5.1. Two-choice experiment ......................................................................................... 78
      2.5.2. No-choice experiment ........................................................................................... 78
      2.5.3. Effect of volatiles on oviposition ......................................................................... 79
   2.6. Y—tube experiment ........................................................................................................ 79
   2.7. Larval bioassays ............................................................................................................. 80
      2.7.1. Two-choice experiment ......................................................................................... 80
      2.7.2. Larval acceptance and transmission of conidia of \textit{B. cinerea} ............................... 80
   2.8. Statistical analysis .......................................................................................................... 81
3. RESULTS .................................................................................................................................. 81
   3.1. Bioassay of oviposition behaviour ................................................................................. 81
      3.1.1. Two-choice experiment ......................................................................................... 81
      3.1.2. No-choice experiment ........................................................................................... 84
   3.2. Effect of volatiles on oviposition ................................................................................... 84
   3.3. Y—tube experiment ........................................................................................................ 84
   3.4. Larval bioassays ............................................................................................................. 85
      3.4.1. Two-choice experiment ......................................................................................... 85
      3.4.2. Larval acceptance and transmission of conidia of \textit{B. cinerea} ............................... 85
4. DISCUSSION ............................................................................................................................ 85
5. CONCLUSION ........................................................................................................................ 95
6. REFERENCES .......................................................................................................................... 95

CHAPTER 4  OVIPPOSITION BEHAVIOUR AND LIFE-HISTORY PERFORMANCE OF \textit{EPIPHYas POSTVITTANA} ON THE LEAVES OF \textit{VITIS VINIFERA} INFECTED WITH \textit{BOTRYTIS CINerea}\textsuperscript{*}

1. INTRODUCTION ...................................................................................................................... 103
2. MATERIAL AND METHODS .............................................................................................. 105
   2.2. Insect culture .................................................................................................................. 105
   2.3. Preparation of conidial suspension .................................................................................. 106
   2.4. Inoculation of \textit{V. vinifera} leaves with \textit{B. cinerea} ...................................................... 106
   2.5. Oviposition behaviour .................................................................................................... 106
      2.5.1. Free-choice experiment .......................................................................................... 106
      2.5.2. Two-choice experiment ......................................................................................... 107
   2.6. Larval development ........................................................................................................ 107
      2.6.1. On \textit{B. cinerea} cultured on potato-dextrose agar .................................................... 107
      2.6.2. On \textit{B. cinerea} cultured on Murashige and Skoog medium .................................... 108
      2.6.3. On \textit{B. cinerea}-infected and uninfected leaves of \textit{V. vinifera} maintained on Knop’s solution ......................................................................................................................... 108
      2.6.4. On \textit{B. cinerea}-infected and uninfected leaves of \textit{V. vinifera} maintained on potato-dextrose agar or Murashige and Skoog medium ................................................................. 110
   2.7. Statistical analysis ........................................................................................................... 112
3. RESULTS .................................................................................................................................. 112
CHAPTER 5  
BOTRYTIS CINEREA INDUCED CHANGES IN VITIS VINIFERA LEAVES INFLUENCE THE OVIPOSITION BEHAVIOUR AND LIFE HISTORY OF EPIPHYAS POSTVITTANA*

1. INTRODUCTION ............................................................................................................ 133
2. MATERIAL AND METHODS ....................................................................................... 135
  2.1. Insect rearing ............................................................................................................ 135
  2.2. Fungus culture and preparation of conidial suspension ........................................ 135
  2.3. Inoculation of V. vinifera leaves with B. cinerea .................................................... 136
  2.4. Headspace collection and volatile profiling ........................................................... 136
  2.4.1. Identification of the compounds ..................................................................... 137
  2.5. Wind-tunnel assay .................................................................................................. 137
  2.6. Larval development ............................................................................................... 138
  2.7. Statistical analysis .................................................................................................. 139
3. RESULTS .................................................................................................................... 140
  3.1. Volatile profile ....................................................................................................... 140
  3.3. Larval development ............................................................................................... 140
4. DISCUSSION.............................................................................................................. 145
  4.1. Botrytis cinerea infection alters the volatile profile of V. vinifera ......................... 145
  4.2. Infection by B. cinerea on V. vinifera does not attract gravid females of E. postvittana.... 146
  4.3. Botrytis cinerea infection of V. vinifera influences the survival rate and larval performance of E. postvittana ................................................................. 147
5. CONCLUSION ............................................................................................................ 149
6. REFERENCES ........................................................................................................... 149

CHAPTER 6  
EPIPHYAS POSTVITTANA – BOTRYTIS CINEREA–VITIS VINIFERA INTERACTION: THE ROLE OF B. CINEREA ON THE DEVELOPMENT OF E. POSTVITTANA IN SYNTHETIC NUTRITIONAL MEDIA*

1. INTRODUCTION ............................................................................................................ 155
2. MATERIALS AND METHODS .................................................................................... 157
  2.1. Insect culture .......................................................................................................... 157
  2.2. Lyophilization of samples of the mycelia of B. cinerea and leaves of V. vinifera.... 157
  2.3. Larval-diet preparation ......................................................................................... 158
  2.4. Larval preference: Two-choice experiment .......................................................... 158
  2.5. Life history performance of E. postvittana: ........................................................... 159
    2.5.1. Growth and development ............................................................................. 159
    2.5.2. Adult performance ......................................................................................... 159
  2.7. F2 generation ........................................................................................................ 160
  2.8. Statistical analysis ................................................................................................ 160
CHAPTER 7 OVIPOSITION PREFERENCE AND LARVAL PERFORMANCE OF EPiphyas Postvittana on Botrytis Cinerea Infected Berries of Vitis Vinifera*

1. INTRODUCTION ................................................................................................................................. 177
2. MATERIALS AND METHODS .............................................................................................................. 179
  2.1. Insect culture .................................................................................................................................. 179
  2.2. Preparation of conidial suspension ................................................................................................. 180
  2.3. Infection of V. vinifera berries by B. cinerea .................................................................................. 180
  2.4. Oviposition behaviour ..................................................................................................................... 180
    2.4.1. Two-choice experiment .............................................................................................................. 180
    2.4.2. No-choice experiment – rate of oviposition ............................................................................. 182
    2.4.3. No-choice experiment – effect of volatiles on oviposition ....................................................... 182
    2.4.4. Olfactory response of adults of E. postvittana ............................................................................ 182
  2.5. Larval behaviour .............................................................................................................................. 183
    2.5.1. Two-choice experiment .............................................................................................................. 183
    2.5.2. Transmission of conidia of B. cinerea ......................................................................................... 183
  2.6. Larval development .......................................................................................................................... 184
    2.7. Statistical analysis ......................................................................................................................... 184
3. RESULTS .............................................................................................................................................. 185
  3.1. Oviposition behaviour ..................................................................................................................... 185
    3.1.1. Two-choice experiment .............................................................................................................. 185
    3.1.2. No-choice experiment – rate oviposition .................................................................................. 185
    3.1.3. No-choice experiment – effect of volatiles on oviposition ....................................................... 186
    3.1.4. Olfactory response of adults of E. postvittana ............................................................................ 186
  3.2. Larval behaviour .............................................................................................................................. 186
    3.2.1. Two-choice experiment .............................................................................................................. 186
    3.2.2. Transmission of conidia of B. cinerea ......................................................................................... 186
  3.3. Larval development .......................................................................................................................... 189
4. DISCUSSION ....................................................................................................................................... 189
  4.1. Females of E. postvittana are deterred from ovipositing on Botrytis cinerea-infected berries of Vitis vinifera .................................................................................................................. 188
  4.2. The larvae of E. postvittana show no significant preference to Botrytis cinerea-infected berries of Vitis vinifera ........................................................................................................................................ 193
  4.3. Botrytis cinerea-infected berries of V. vinifera affect the survival and life-history performance of E. postvittana larvae .................................................................................................................. 190
5. CONCLUSION ..................................................................................................................................... 197
6. REFERENCES ....................................................................................................................................... 197
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.</td>
<td>Fungus culture and preparation of conidial suspension</td>
<td>207</td>
</tr>
<tr>
<td>2.3.</td>
<td>Infection of <em>V. vinifera</em> berries with <em>B. cinerea</em></td>
<td>208</td>
</tr>
<tr>
<td>2.4.</td>
<td>Headspace collection and volatile profiling</td>
<td>208</td>
</tr>
<tr>
<td>2.4.1.</td>
<td>Identification of the compounds</td>
<td>208</td>
</tr>
<tr>
<td>2.5.</td>
<td>Wind-tunnel assay</td>
<td>209</td>
</tr>
<tr>
<td>2.6.</td>
<td>Oviposition behaviour</td>
<td>210</td>
</tr>
<tr>
<td>2.6.1.</td>
<td>Two-choice experiment</td>
<td>210</td>
</tr>
<tr>
<td>2.6.2.</td>
<td>Laboratory standard volatiles in the presence of uninfected <em>V. vinifera</em> berries</td>
<td>210</td>
</tr>
<tr>
<td>2.6.3.</td>
<td>Laboratory standard volatiles in the absence of uninfected <em>V. vinifera</em> berries</td>
<td>210</td>
</tr>
<tr>
<td>2.7.</td>
<td>Statistical Analysis</td>
<td>211</td>
</tr>
<tr>
<td>3.</td>
<td>RESULTS</td>
<td>211</td>
</tr>
<tr>
<td>3.1.</td>
<td>Volatiles profile</td>
<td>211</td>
</tr>
<tr>
<td>3.2.</td>
<td>Wind tunnel assay Oviposition behaviour</td>
<td>212</td>
</tr>
<tr>
<td>3.3.</td>
<td>Oviposition behaviour</td>
<td>208</td>
</tr>
<tr>
<td>3.3.1.</td>
<td>Two-choice experiment</td>
<td>212</td>
</tr>
<tr>
<td>3.3.2.</td>
<td>Laboratory standard volatiles in the presence of <em>V. vinifera</em> berries</td>
<td>212</td>
</tr>
<tr>
<td>3.3.3.</td>
<td>Laboratory standard volatiles in the absence of berries of <em>V. vinifera</em></td>
<td>214</td>
</tr>
<tr>
<td>4.</td>
<td>DISCUSSION</td>
<td>215</td>
</tr>
<tr>
<td>4.1.</td>
<td><em>Botrytis cinerea</em> infection changes the volatile profile of <em>V. vinifera</em></td>
<td>215</td>
</tr>
<tr>
<td>4.2.</td>
<td>Volatiles from <em>B. cinerea</em> infected berries inhibit attraction and oviposition of <em>E. postvittana</em></td>
<td>2226</td>
</tr>
<tr>
<td>5.</td>
<td>REFERENCES</td>
<td>224</td>
</tr>
<tr>
<td>6.</td>
<td>REFERENCES</td>
<td>224</td>
</tr>
</tbody>
</table>

CHAPTER 9  GENERAL DISCUSSION AND CONCLUSION

1. GENERAL DISCUSSION | 230 |
| 1.1. Chardonnay the most susceptible variety to *E. postvittana* in Central West New South Wales, Australia | 231 |
| 1.2. *Botrytis cinerea* infection influences the bionomics of *E. postvittana* | 232 |
| 1.2.1. *Botrytis cinerea* infection alters the oviposition behaviour of *E. postvittana* | 232 |
| 1.2.2. *Botrytis cinerea* alters the behaviour of *E. postvittana* larvae | 235 |
| 1.2.3. *Botrytis cinerea* infection alters *E. postvittana* life-history | 237 |
| 1.3. Three-way interacting system | 240 |
| 2. CONCLUSION | 242 |
| 3. REFERENCES | 2436 |
Certificate of Authorship

I hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis [or dissertation, as appropriate]. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

I agree that this thesis be accessible for the purpose of study and research in accordance with normal conditions established by the Executive Director, Library Services, Charles Sturt University or nominee, for the care, loan and reproduction of thesis, subject to confidentiality provisions as approved by the University.

<table>
<thead>
<tr>
<th>Name</th>
<th>Syed Zulfiqar Mehdi Rizvi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>21/10/2016</td>
</tr>
</tbody>
</table>
Acknowledgments

First and above all, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed successfully.

I wish to express my sincere thanks to Anantanarayanan Raman, my principal supervisor, for his guidance and encouragement through the project, which helped me developing a professional approach towards scientific research. I am thankful to Warwick Wheatley and Geoffrey Cook for their associate-supervisory role until October 2014.

I sincerely acknowledge the support provided by Karyn Snare (Administration Assistant, School of Agricultural & Wine Sciences [SAWS], CSU, Orange), help rendered by Karen Gogala and Jeff West (Laboratory Managers, SAWS, CSU, Orange) who helped me in CSU Orange research laboratory. The statistical analysis presented in this thesis was done in consultation with Helen Nicol (Biometrician, CSU). I thank her for the support. I extend my thanks to Gayle Smythe (Associate Dean, Research, Honours & Graduate Studies, Faculty of Science, CSU) for supporting my extension application, which enabled me to complete my thesis in its present form.

I am thankful to Charles Hocart and Thy Truong (Mass Spectrometry Officers — Mass Spectrometry Facility, Australian National University, Canberra), Abdel Qader Qawasmeh (Hebron University, Palestine) for their help and assistance in gas chromatography work.

I am deeply thankful to Peter Headberg (Vineyard Manager, Hedberg Hill, Orange, NSW), Clayton Kiely (Vineyard Manager, Tamburlaine, Borenore, NSW), Sam Statham (Vineyard Manager, Rosnay, Canowindra, NSW), and Justin Jarrett (Vineyard Manager, See Saw Wine, Orange, NSW) for allowing me to use their vineyards for field observations.
I am grateful to Michael Priest (Department of Primary Industries, Orange, New South Wales) for identifying fungal materials I am thankful to Maryam Yazdani and Feng Yi (Waite campus, University of Adelaide, SA) for providing the eggs of E. postvittana.

I deeply thank to all my friends especially Anwar Nawaz Khan, Anamika Sharma and Muhammad Bhuiyan for their support, thus making my study time enjoyable. I could not be able to complete this long journey without the help of my family. I am thankful to my family members for the support they offered me throughout my study.
Context of the thesis

This thesis embodies a study of chemical-ecological interactions of *Epiphyas postvittana* with *Vitis vinifera* leaves and berries that were infected by *Botrytis cinerea*. Experiments were carried out from July 2012 to September 2015 fulfilling the requirements of a Ph.D. degree from Charles Sturt University, New South Wales.

This thesis consists of nine chapters: Chapter 1 is General Introduction, 3—8 refer to the experimental trials, and 9 General Discussion and Conclusion. My papers published in *Australian Journal of Grape and Wine Research* (Adelaide, Australia), *Austral Entomology* (Darwin, Australia), *Vitis — Journal of Grapevine Research* (Braunschweig, Germany), *Journal of Economic Entomology* (Washington, D.C., U.S.A.), *Insect Science* (Beijing, People’s Republic of China), and *Entomologia Experimentalis et Applicata* (Wageningen, The Netherlands) constitute Chapters 2, 3, 4, 6, 7 and 8, respectively. The text forming Chapter 5 has been communicated to *Ethology, Ecology and Evolution* (Firenze, Italy), which is currently under review.

In Australia, *E. postvittana* infests certain varieties such as, Chardonnay, Sémillon, and Sauvignon Blanc, more intensely than the others, such as, Shiraz, Cabernet Sauvignon, and Merlot (Paull, 2008; Weeks & Pitman, 2012; Baker, 2005). Many of the vineyard managers in central-west New South Wales believe that Chardonnay is the most susceptible to *E. postvittana* and Marsanne is the least. *Epiphyas postvittana* shows flexibility in its behavioural, physiological, and demographic performances, while responding to environmental heterogeneity (Gu & Danthanarayana, 2000). Considerable differences in life-history traits and population-growth parameters within and among populations have been shown (Gu & Danthanarayana, 2000). Chapter 2 discusses the findings on the life-history performance of *E. postvittana* on five different varieties of *V. vinifera* viz., Chardonnay, Sauvignon Blanc, Merlot, Marsanne, and Sémillon.
The larvae of *E. postvittana* occur with *B. cinerea*-infected berries and leaves of *V. vinifera*. Feeding damage by *E. postvittana* larvae renders *V. vinifera* susceptible to infection by *Botrytis cinerea*, which induces the ‘grey mould’ disease (Lo & Murrell, 2000). *Botrytis cinerea* is a ubiquitous necrotrophic fungus that occurs on both leaves and berries of *V. vinifera*. In Australian vineyards, *E. postvittana* adults usually encounter *B. cinerea* during oviposition, whereas their larvae concurrently exist with *B. cinerea* feeding on their hyphae and conidia, thus establishing a three-way interacting system (Bailey et al., 1996). *Botrytis cinerea* infection is known to alter the chemistry of its host and consequently influence the population dynamics of Lepidoptera (Mondy et al., 1998; Tasin et al., 2011). Chapters 3–8 discuss the role of *B. cinerea* infection on the oviposition behaviour, larval olfactory behaviour, and life-history performance of *E. postvittana*.

Chapter 9 — general discussion — analyzes and synthesizes the results found, pulling them into a unifying thread.

To conform to a consistent style, Chapters 2—8 have been editorially amended in such a way that the general presentation details, such as formatting, arrangement of figure legends, and style of ‘in-text’ and ‘end-list’ references, thus providing reading comfort to the examiners of this thesis. The emendations made have been referred as ‘minor modifications’ in cover pages of each chapter. I reiterate that no major change has been made to the published results and other technical details.

REFERENCES


Abstract

In this thesis, I present the detail of (i) impact of varietal differences of \textit{V. vinifera} on the infestation of \textit{E. postvittana}, (ii) volatile composition of \textit{B. cinerea}-infected and uninfected leaves and berries of \textit{V. vinifera}, (iii) impact of \textit{B. cinerea} on the bionomics of \textit{E. postvittana}.

To provide a comprehensive context, Chapter 1 includes a review of earlier literature in the areas of (i) insect–fungus–plant interaction: three-way interacting system, (ii) theories explaining such interactions, (iii) bionomics of \textit{E. postvittana}, and (iv) bionomics of \textit{B. cinerea} on \textit{V. vinifera}. Against these contextual notes, I have outlined the purpose and goals of this study.

Chapter 2 evaluates larval and adult-reproductive performances of \textit{Epiphyas postvittana} by testing them against the foliar chemistry of five varieties of \textit{V. vinifera}. I determined total nitrogen, carbohydrates, phenols, and nutrients (cations) in the leaves of Chardonnay, Sauvignon Blanc, Merlot, Sémillon and Marsanne of \textit{V. vinifera}. The life-history performance of \textit{E. postvittana} was evaluated by rearing \textit{E. postvittana} from eggs on chosen varieties of \textit{v. vinifera}. Nitrogen content of Chardonnay was significantly greater than the other tested varieties. Different varieties of \textit{V. vinifera} influenced the growth factors, such as developmental time of the larvae and female pupal mass, variously. Adult-reproductive performance, measured as adult-male longevity and fecundity rates, and fertility among females varied, when reared on nutrient media incorporated with freeze-dried leaf materials of the selected varieties of \textit{V. vinifera}. Leaves of Chardonnay include the greatest level of nitrogen content and were found to be the most suitable for the best larval performance of \textit{E. postvittana}. Fitness-index study showed that the female-reproductive performance was significantly influenced, when raised on Chardonnay, whereas, when raised on Marsanne adults performed poorly. Among the tested varieties, Chardonnay proved to be the ideal host (Rizvi & Raman, 2016a).

Chapter 3 explains oviposition preference of adults and feeding preference of larvae of \textit{E. postvittana} larvae towards the uninfected and \textit{B. cinerea}-infected leaves
of *V. vinifera*. I used three different levels of infection [mild (5–10%), moderate (30–60%) and intense (90–100%)] to measure the oviposition behaviour and feeding preference of *E. postvittana*. The host-seeking gravid females of *E. postvittana* ‘tested’ the infection status of the host plant using olfactory, visual, and tactile cues; in consequence, they laid significantly fewer eggs on the moderately (30–60%) and intensely (90–100%) infected leaves as against the uninfected leaves of *V. vinifera*. Neonate larvae preferred to feed on mildly and moderately infected leaves, as against the uninfected (control) leaves, and showed no preference for the intensely infected leaves. External and internal examination of the larvae established that the larvae fed on *B. cinerea*-infected leaf. Viable conidia of *B. cinerea* occurred both on the body surface and within the gut of the neonate larvae (Rizvi *et al.*, 2015a).

Chapter 4 evaluates how the infection of the leaves of *V. vinifera* by *B. cinerea* influences the life-history performance of larvae and the oviposition behaviour of *E. postvittana*. Free-choice and two-choice experiments were conducted to test the oviposition behaviour of gravid *E. postvittana* towards uninfected and *B. cinerea*-infected (10–30%) leaves of *V. vinifera*. The effects of *B. cinerea*-infected leaves of *V. vinifera* on the growth and development of *E. postvittana* were also characterized. The oviposition preference of *E. postvittana* was strongly influenced by the olfactory and tactile cues. Volatiles from *B. cinerea*-infected plants significantly deterred oviposition and in consequence, adult females laid fewer eggs on *B. cinerea*-infected leaves of *V. vinifera* than on uninfected leaves. The mortality rate of larvae fed on *B. cinerea*-infected leaves were not significantly different from the larvae fed on uninfected leaves of *V. vinifera*. Whereas, the larvae of *E. postvittana* fed on *B. cinerea*-infected leaves had significantly shorter developmental period, attained heavier pupal mass, and on becoming adults they laid greater numbers of eggs than the larvae that were enabled to feed on uninfected leaves of *V. vinifera*. The larvae of *E. postvittana* was reared on exclusive-fungus diet but all larvae died before pupation indicating that for a better larval performance and adult reproductive output of *E. postvittana*, the *V. vinifera—B. cinerea* interacting system is but imperative (Rizvi & Raman, 2015a).

Chapter 5 discusses the changes in volatile composition of leaves of *V. vinifera* after infection by *B. cinerea* and its consequences on the oviposition
behaviour of *E. postvittana*. The key volatiles from *B. cinerea*-infected *V. vinifera* leaves were 2-hexene-1-ol, 2-hexenal (E), 1-hexanol, 3-octanone, and 1-octen-3-ol, whereas the same leaves included highly reduced levels of nonanal, benzaldehyde, acetic acid, and hexanal. Results from the wind-tunnel experiments showed that gravid females avoid infected leaves, pointing to that the newly generated 2-hexene-1-ol, 2-hexenal (E), 1-hexanol, 3-octanone, and 1-octen-3-ol and low levels of nonanal, benzaldehyde, acetic acid, and hexanal signal the non-suitability of the infected *V. vinifera* leaves to gravid females of *E. postvittana*, thus preventing oviposition. This was further confirmed by the total failure of my attempts to rear neonate larvae to adult on *B. cinerea*-infected leaves (70%) (Rizvi & Raman, 2016b).

Chapter 6 includes the preference and performance of the larvae of *E. postvittana* raised solely on a synthetic diet (Diet A) and synthetic diet incorporated with the mycelial material of *B. cinerea* (Diet B). To characterize the effect of fungus on the development of *E. postvittana*, another synthetic diet was prepared that included the lyophilized leaf material of *V. vinifera* (Diet C). When raised on Diets B and C, a decrease in the duration of larval development and an increase in the survival and fecundity rate of *E. postvittana* occurred. Diet B influenced the pupal mass, but a significant increase occurred when the larvae were fed on Diet C. The larval emergence rate was the greatest in *E. postvittana* raised on Diet B, followed by those on Diet C. The F2 generation of the larvae reared on Diet B showed similar effects as F1 on the life-history performance of the larvae. Diet B enhanced the life-history performance of *E. postvittana*, although the larvae of *E. postvittana* showed little preference to Diet B. The greater fertility rate of *E. postvittana* reared on Diet B suggests the importance of sterols (e.g., cholesterol), which serve as precursors to different ecdysteroids that regulate many critical processes through embryonic development (Rizvi & Raman, 2015b).

Chapter 7 tests Jaenike’s preference—performance hypothesis (also referred to as the ‘mother-knows-the-best’ hypothesis), which states that the oviposition preference should correspond with host suitability for offspring development; the adult females, thus maximize the fitness of their offspring by ovipositing at the most appropriate host sites. I tested the effect of volatiles from *B. cinerea*-infected berries
and uninfected (control) berries of *V. vinifera* on the oviposition behaviour of *E. postvittana*. I also characterized the effects of infection of *V. vinifera* berries with *B. cinerea* on the growth and development of *E. postvittana*. Contrary to the preference—performance hypothesis, oviposition choices made by gravid *E. postvittana* did not result in the best offspring survival, development, and performance. The preference for oviposition by *E. postvittana* was strongly influenced by the olfactory and tactile cues. She laid fewer eggs on *B. cinerea*-infected berries compared to uninfected berries of *V. vinifera*. The larvae of *E. postvittana* showed no preference to uninfected berries of *V. vinifera*. The larvae fed on *B. cinerea*-infected berries of *V. vinifera* showing greater survival rate, shorter time to pupation, greater pupal mass, and on becoming adults they laid more numbers of eggs than the larvae that were enabled to feed on uninfected berries. The larvae of *E. postvittana* transport the conidia of *B. cinerea* and transmit grey-mould disease to uninfected berries of *V. vinifera* (Rizvi et al., 2015b).

Chapter 8 determines the volatiles composition of *B. cinerea*-infected berries of *V. vinifera* and its impact on oviposition behavior of *E. postvittana*. The effect of synthetic volatiles (ethanol and 3-methyl-1-butanol) on the oviposition behavior of *E. postvittana* was also measured. The volatiles emanating from *B. cinerea*-infected berries did not significantly attract gravid females of *E. postvittana* and in consequence significantly fewer eggs on infected berries were occurred. The infection of *B. cinerea* elicited a substantial reduction in attraction of females of *E. postvittana* during the wind tunnel bioassay. Alcohols such as ethanol and 3-methyl-1-butanol were abundant in *B. cinerea*-infected berries. In oviposition bioassays with synthetic compounds, ethanol and 3-methyl-1-butanol confirmed the essential function of olfactory cues in this process (Rizvi & Raman, 2016c).

Chapter 9 is the general discussion of larval and adult-reproductive performances of *Epiphyas postvittana* on five different varieties of *V. vinifera*. The life-history performance of *E. postvittana* was significantly improved, when reared on Chardonnay, whereas, when reared on Marsanne that declined. The results show that Chardonnay is the most susceptible to *E. postvittana* and Marsanne least. Chapter 9 further discusses the influence of *B. cinerea* infection on the oviposition behaviour, larval olfactory behaviour, and life-history performance of *E. postvittana*. 
Infection of *V. vinifera* by *B. cinerea* alters the volatiles composition of leaves and berries of *V. vinifera*, which consequently influenced the olfactory behaviour of larvae and oviposition behaviour of *E. postvittana*. In leaves, low infection level improves the larval development and reproductive outputs, whereas the intense infection level has detrimental effect on life-history performance of *E. postvittana*. *Botrytis cinerea*-infected berries of *V. vinifera* positively influence the larval development and reproductive performance of *E. postvittana*. Olfactory cues along with tactile cues play a key role in oviposition behaviour.

In Australian vineyards, *E. postvittana* adults frequently encounter *B. cinerea* during oviposition, and *E. postvittana* larvae occur along with *B. cinerea* feeding on their hyphae and spores, establishing a three-way interacting system. The infection of *V. vinifera* by *B. cinerea* alters the oviposition behaviour and influence the life-history performance of *E. postvittana*. The co-occurrence of the *B. cinerea* and larvae of *E. postvittana* on *V. vinifera* and the improved life-history performance of *E. postvittana* fed on *B. cinerea*-infected *V. vinifera* indicate a possible mutualism, which remains to be verified. Although, *B. cinerea* infection influenced key life-history parameters, of *E. postvittana* the present study do not support that the fungus involvement is critical for the survival and growth of *E. postvittana*.

REFERENCES


Rizvi, S.Z.M. and Raman, A. (2015b) *Epiphyas postvittana* (Lepidoptera: Tortricidae)—*Botrytis cinerea* (Helotiales: Sclerotiniaceae)—*Vitis vinifera*


Chapter 1

General Introduction

*Epiphyas postvittana–Vitis vinifera–Botrytis cinerea*: three-way interacting system
This page is intentionally left blank
1. GENERAL INTRODUCTION

1.1. Insect–fungus–plant interaction: three-way interactions

1.1.1. Insect–plant interactions

In natural ecosystems, plants and insects continuously interact in complex ways. The association between them could be intimate that some of the interacting plants gain, e.g., via pollination. Plants, in turn, provide shelter, oviposition sites, and food for the interacting insects (Schoonhoven et al., 2005). In several instances, the interaction between plants and insects appears so impressive that people consider them to have co-evolved (e.g. Scriber, 2002). The example of *Salvia* (Lamiaceae)–Anthophorini (Hymenoptera: Apidae) interaction powerfully illustrates such an intimate association, impressing as a co-evolved relationship (Westerkamp & Claßen-Bockhoff, 2007).

Growth and reproduction of plant-feeding insects depend on the quality of plants, which the insects utilize. Plant quality ultimately refers to the chemical composition of the plant, such as levels of nitrogen, carbon, trace elements, and secondary metabolites that either positively or negatively influence the performance of feeding insects. For example, the fecundity of *Drepanosiphum platanoidis* (Hemiptera: Aphididae) depends on the amino-acid levels in phloem of *Acer pseudoplatanus* (Sapindales: Sapindaceae) (Awmack & Leather, 2002). Plant carbohydrates also play a major role in the performance of feeding insects. An increase in carbohydrate levels in artificial diets leads to a decreased fecundity of *Melanoplus sanguinipes* (Orthoptera: Acrididae), whereas that of *Phoetiotes nebrascensis* (Orthoptera: Acrididae) remains unaltered (Joern & Behmer, 1998). Lipids and sterols also play a key role in insect performance. Sterols can act as phagostimulants. For example, artificial diets containing low sterol levels influence the survival rate of *Plutella xylostella* (Lepidoptera: Plutellidae) by reducing fecundity and fertility (Behmer & Grenbenok, 1998).
1.1.2. *Fungal association in insect–plant interactions*

Plant-feeding insects may compensate for low nutrient level of a plant by either increasing the volume of consumption of plant tissues (Behmer & Nes, 2003) or facilitating symbiotic relationship with microbes (Baumann *et al*., 1997). In the natural environment, organisms such as insects, mites, fungi, and bacteria remain associated with plants, sometimes closely and sometimes distantly. Among such interactions, insects and fungi are associated with many plants at the same time, establishing three-way interactions. Such interactions involving plants, insects, and microbes can have consequences on the performance of one or the other (Hatcher, 1995; van Dam, 2009). For example, microbes can influence the performance of plant-feeding insect and *vice versa* (Tack & Dicke, 2013). The microbes can influence the performance of insects, especially when the microbes live within them as symbionts (Ferrari & Vavre, 2011; White, 2011). Microbes, when associated with plants, alter the nutritional quality of plants, leading to either positive (Friedli & Bacher, 2001; Cardoza *et al*., 2003; Mondy & Corio-Costet, 2004; Tasin *et al*., 2011) or negative (Hatcher *et al*., 1994; Lappalainen *et al*., 1995; Tinney *et al*., 1998; Stout *et al*., 1999; Rostás & Hilker, 2002) effects on the fitness of plant-feeding insects. Occasionally neutral effects have also been known in similar contexts (Apriyanto & Potter, 1990; Ajlan & Potter, 1991; Stout *et al*., 1999).

In natural conditions, insects also operate as vectors of plant pathogens. In the initiation phase of a disease, a temporal synchronization among a wounded plant, viable microbial spores, and moisture is necessary for the infection to set in (Coertze & Holz, 2002). Some insects transmit spores among plants and to sites on plants and by their feeding action inflict damage to plants, thus creating wounds, *e.g.* *Ips pini* (Coleoptera: Curculionidae) transmits spores of *Sphaeropsis sapinea* (Botryosphaeriales: Botryosphaeriaceae) to *Pinus nigra* (Pinales: Pinaceae) inducing top-dieback disease to *P. nigra* (Whitehill *et al*., 2007). Insects can act both as the suppliers of microbial inoculum onto wounds and also as initiators of disease (Engelbrecht, 2002). Inocula consisting conidia and/or mycelia could be deposited incidentally on plant wounds by insects (Coertze & Holz, 2002; Engelbrecht, 2002). For example, the conidia of *Botrytis cinerea* (Heliotiales: Sclerotiniaceae) occur trapped between body segments and among body hairs of *Drosophila melanogaster*
(Diptera: Drosophilidae) (Louis et al., 1996), Lobesia botrana (Lepidoptera: Tortricidae) (Fermaud & Le Menn, 1989), Thrips obscuratus (Thysanoptera: Thripidae) (Fermaud & Gaunt, 1995), Ceratitis capitata (Diptera: Tephritidae) (Engelbrecht, 2002), and Epipyas postvittana (Lepidoptera: Tortricidae) (Bailey et al., 1996), which are deposited on plants during feeding. Drosophila melanogaster transmits conidia onto the surface of Vitis vinifera berries during feeding and also via faeces (Engelbrecht, 2002).

Botrytis cinerea outbreaks in European vineyards are usually associated with L. botrana. The first generation of L. botrana damages flowers, the second unripe berries (Fermaud & Giboulot, 1992), and the third ripe berries (Fermaud & Le Menn, 1992). While feeding on V. vinifera tissues, the larvae of L. botrana carry the conidia of B. cinerea not only on their body segments but also in their digestive tract because they consume fungal material along with V. vinifera tissues. Conidia of B. cinerea carried externally as well as those ingested remain viable for a reasonable period of time enabling their germination and consequent infection of V. vinifera berries (Fermaud & Le Menn, 1989). Infected berries act as conidial reservoirs supplying spores to generations of larvae of L. botrana (Fermaud & Giboulot, 1992). Feeding on infected berries consistently improved the fecundity rate and life-history performance of L. botrana (Mondy & Corio-Costet, 2000) indicating that a mutualistic relationship possibly exists between L. botrana and B. cinerea on V. vinifera (Mondy et al., 1998). In the interaction involving E. postvittana–V. vinifera–B. cinerea, a similar pattern is apparent (Bailey et al., 1996). In southeast-Australian vineyards, both spring (September–November) and summer (December–February) generations of E. postvittana are associated with B. cinerea (Bailey et al., 1996). The spring generation of E. postvittana larvae often coincides with flowering of V. vinifera, indicating that the larvae possibly transmit the conidia of B. cinerea either from leaves to flowers or between flowers triggering a latent infection of berries (Bailey et al., 1997).
Fungal infection can alter the chemistry of plants and influence larval performance of insects feeding on the plants (Mondy & Corio-Costet, 2000; Raman et al., 2012). Fungal infection can either increase the concentration of defence compounds or deteriorate the nutritional quality of the plant and thus influence plant-feeding insect’s development negatively (Tasin et al., 2012). For example, *Gastrophysa viridula* (Coleoptera: Chrysomelidae) shows significantly low fecundity, when fed on *Uromyces rumicis* (Pucciniales: Pucciniaceae) infected leaves of *Rumex crispus* and *R. obtusifolius* (Caryophyllales: Polygonaceae) (Hatcher et al., 1994). The larvae of *Epirrita autumnata* (Lepidoptera: Geometridae) experience detrimental effect on life-history parameters, when fed on the *Melampsoridium betulinum* (Pucciniales: Pucciniastraceae) infected leaves of *Betula pubescens* (Fagales: Betulaceae) (Lappalainen et al., 1995). *Spodoptera frugiperda* (Lepidoptera: Noctuidae) reared on *Lolium perenne* (Poales: Poaceae) infected with *Neotyphodium lolii* (Hypocreales: Clavicipitaceae) show reduced larval mass and delayed development compared with the larvae reared on uninfected *L. perenne* (Hardy et al., 1985). Infection of plants by pathogenic fungi can increase the levels of various secondary metabolites, enabling defence (Busam et al., 1997; Derckel et al., 1999). For example, in response to *B. cinerea*, leaves of *V. vinifera* accumulate secondary metabolites, pathogenesis-related proteins, chitinase, and β-1,3–glucanase (Trotel-Aziz et al., 2006). In contrast, fungal infection can suppress the plant’s defence capacity against insects by modifying its secondary metabolism on the one hand (Thaler et al., 1999) and improving nutritional quality on the other, thus rendering the plant susceptible for insect colonization (Cardoza et al., 2003). For example, the larvae of *Tischeria ekebladella* (Lepidoptera: Tischeriidae) show an increased growth rate when fed on leaves of *Quercus robur* (Fagales: Fagaceae) infected by *Erysiphe alphitoides* (Erysiphales: Erysiphaceae) (Tack et al., 2012). Cardoza et al. (2002) explain that the survival and pupation rates of *Spodoptera exigua* (Lepidoptera: Noctuidae) increased when fed on the foliage of *Arachis hypogaea* (Fabales: Fabaceae) infected by *Sclerotium rolfsii* (Atheliales: Atheliaceae), which includes high levels of soluble sugars and low levels of starch and total-soluble
phenolics. Several insect species utilize fungal mycelia and spores during feeding on infected-plant tissues indicating that the fungal material can also be a source of nutrition, demonstrated in a study of *L. botrana−V. vinifera−B. cinerea* interaction in simulated conditions (Mondy *et al*., 1998). With the larvae of *L. botrana* fed on an artificial diet that included the *B. cinerea* material, the larvae exhibit a higher rate of survival, faster development, and increased fecundity (Mondy & Corio-Costet, 2004).

Unlike most vertebrates, insects lack the ability to synthesize sterols necessary as precursors to hormones that are required in bio-systems as regulators of developmental processes (Behmer & Nes, 2003). Plant-feeding insects acquire sterols or sterol precursors from plants and/or from the microbial symbionts (Behmer & Nes, 2003). Specific taxa belonging to the Psocoptera, Thysanoptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera, and Isoptera have been shown to derive their cholesterol requirement from fungi. Plants include a variety of lipids (e.g., phospholipids, sterols), which enable storing of energy in cell membranes (Schmid & Ohlrogge, 2002). Plants usually include low levels of cholesterol, which are generally inadequate for the development of plant-feeding insects (Behrman & Gopalan, 2005). Nevertheless, such insects use available steroid precursors that exist as sterols in plants. Sitosterol is the most commonly occurring plant sterol, which supports the growth and development in plant-feeding insects (Behmer & Nes, 2003). Stigmasterol is another commonly occurring plant sterol, although it usually occurs at lower levels than sitosterol. Stigmasterol is also readily used by many plant-feeding insects, particularly the Lepidoptera.

In general, ergosterol predominantly occurs in fungi, and most insects symbiotic with fungi have the ability to acquire ergosterol from them and metabolize it to cholesterol (Svoboda *et al*., 1995; Behmer & Nes, 2003). In several insect–fungus symbioses, the insect depends on sterols provided by the fungus (Behmer & Nes, 2003). These include species of Scolytinae (Coleoptera) that feed solely on fungi they grow as galleries in wood (Bentz & Six, 2006). The Scolytinae are dependent on ergosterol produced by their fungal symbionts for successful oocyte development, oviposition, larval development, and pupation (Bentz & Six, 2006). *Hypothenemus hampei* (Coleoptera: Curculionidae) feeds on the berries of
Coffea arabica (Gentianales: Rubiaceae), but cannot either moult or reproduce without ergosterol from Fusarium solani (Hypocreales: Nectriaceae) that occurs on C. arabica (Morales-Ramos et al., 2000).

1.1.4. Fungal infection alters the oviposition preference of plant-feeding insects

One critical mediator in plant–insect interactions is the availability of chemical cues emitted as volatiles (Schoonhoven et al., 2005; Bruce et al., 2010). Plant-feeding insects use sensory cues, such as olfactory, contact chemoreceptive, and visual, to search and detect food, mates, predators and parasitoids, competitors, and locate oviposition sites (Wyatt, 2003; Schoonhoven et al., 2005). Compared with vision and contact chemoreception, olfaction, in particular, plays a key role in the assessment of plant by recognizing organic volatiles (e.g., Beyaert et al., 2010). Insects possess specialized olfactory organs that enable them with a remarkable sensory sensitivity and specificity (Hansson, 1999; Hallem et al., 2006). Volatile cues transmit encoded information about the quality of the potential host. Several infective fungi can modify the chemistry of the plant and consequently change the volatile profile (Cosse et al., 1994; Witzgall et al., 2012). For example, infection by Alternaria brassicae (Pleosporales: Pleosporaceae) on Brassica rapa (Brassicales: Brassicaceae) seedlings induces the release of volatile compounds arising from glucosinolate degradation (Doughty et al., 1996). Also, A. hypogaea infected with S. rolfsii release E=4, 8-dimethyl-1,3,7-nonatriene, methyl salicylate, and (E,E)−4,8,12-trimethyl-1,3,7,11−tridecatetraene (Cardoza et al., 2002). Changes in plant-emitted odour can be perceived by an insect, which in turn regulates its oviposition behaviour (Dötterl et al., 2009; Tasin et al., 2011).

Plant-feeding insects show varied behavioural responses, depending on the nature of volatile compounds, which arise during interactions between plants and fungi. Such volatiles can act as either attractants (Mondy et al., 1998, b; Friedli & Bacher, 2001; Shapiro et al., 2012) or deterrents (Tinney et al., 1998; Kluth et al., 2001; Rostás & Hilker, 2002) for the insect in three-way interactions. Consequently, pathogenic fungi may influence the populations of plant-feeding insects. For example, L. botrana shows a preference to yeast (various species of Saccharomyces)
or \emph{B. cinerea} infected berries against uninfected berries of \emph{V. vinifera} (Mondy et al., 1998; Tasin et al., 2011). \emph{Drosophila melanogaster} shows a significantly greater attraction towards \emph{Saccharomyces cerevisiae} infected berries than uninfected berries of \emph{V. vinifera}. Gravid females of \emph{S. exigua} prefer to oviposit on \emph{S. rolfsii} infected leaves and avoid uninfected leaves of \emph{A. hypogaea} (Cardoza et al., 2003). On the other hand, several plant-feeding insects avoid infected parts and prefer uninfected parts of the plants. For instance, when \emph{Microbotryum violaceum} (Microbotryales: Microbotryaceae) infects \emph{Silene latifolia} (Caryophyllales: Caryophyllaceae), \emph{M. violaceum} decreases the synthesis of volatiles that play a role in attracting \emph{Hadena bicruris} (Lepidoptera: Noctuidae). \emph{Phaedon cochleariae} (Coleoptera: Chrysomelidae) avoid feeding and oviposition on \emph{A. brassicaceae} (Pleosporales: Pleosporaceae) infected \emph{B. rapa} leaves and prefer uninfected leaves (Rostás & Hilker, 2002).

\section*{1.2. Theories that explain such interactions}

In principle, oviposition behaviour is interpreted by applying preference—performance, selfish mother, and transmissive mother hypotheses.

\subsection*{1.2.1. Preference—performance hypothesis}

This describes a form of maternal investment whereby females oviposit selectively at sites, which contribute positively to their offspring performance. In this context, ‘preference’ refers to the hierarchical ordering of different types of oviposition sites by females during oviposition, with ‘specificity’ describing the number of types of oviposition sites that a species will utilize. Originally this hypothesis developed from the ‘optimal oviposition theory’ (also known as ‘naive adaptationist’ and ‘mother knows best’ theories) proposed by Jaenike (1978). The preference—performance hypothesis has been extensively tested on diverse insects (Bovill et al., 2013). This hypothesis, when extended to terrestrial plant-feeding insects, relates preference and performance to the nutritional quality of plants, because plant diets of the larvae and other ecological interactions (e.g. predation,
parasitism, competition, and mutualism) regulate the adoption of a range of preference–performance relationships (Gripenberg et al., 2010; Refsnider & Janzen, 2010).

According to ‘optimal oviposition theory’ oviposition preference of a gravid female should match with the host suitably for offspring development. Adult females thus maximize the fitness of their offspring by ovipositing at the most appropriate site on the plant. To maximize insect fitness, this hypothesis predicts a positive correlation between oviposition preference and offspring performance. For example, gravid females of Heliconius eratophyllis (Lepidoptera: Nymphalidae) prefer to lay eggs on Passiflora capsularis (Malpighiales: Passifloraceae) among other multiple congeners, thus maximizing the fitness of her offspring (Ramos et al., 2012). That gravid females often show a preference for oviposition among different plants but the oviposition preference must match with the performance of their offspring is currently debated (Mayhew, 1997; Nyman et al., 2011).

1.2.2. Selfish-motherhood hypothesis

Jaenike (1986) proposed that optimal foraging may determine host choice, as female insects can maximise fitness through the optimisation of adult performance (the realized fecundity). He hypothesised that females may select those hosts that are optimal for adult nutrition instead of those that are optimal for their offspring.

Among plant-feeding insects, optimal foraging and oviposition are mutually dependent; but optimal foraging influences selection of the host more than optimal oviposition (Scheirs et al., 2004). Scheirs et al., (2000) suggest that variation in the adult preference correlates with adult performance rather than with offspring performance in host-plant selection. Optimal foraging has the potential to influence plant choice, because many plant-feeding insects do not only use plants as larval food, but also as adult food (Scheirs & De Bruyn, 2002). For example, Chromatomyia nigra (Diptera: Agromyzidae) (Scheirs et al., 2000) and Altica carduorum (Coleoptera: Chrysomelidae) (Scheirs & De Bruyn, 2002) oviposit where they feed, and they feed on plants that best contribute to adult performance
rather than that of the offspring. Thus, some plant-feeding insects seem to be ‘bad’  
(sensu Mayhew, 2001) mothers.

1.2.3. Transmissive-mother hypothesis

In some instances, maternal choice benefits neither offspring nor parent. For  
example, transmission of some pathogens from mother to offspring (Bernardo,  
1996) or exposure to toxicants can produce offspring that have little or no potential  
to survive (Wiklund & Sundelin, 2001). Rossiter (1996) suggests that the pathogens  
that co-evolve with their hosts are adapted to be transmitted through generations  
despite the fact that such transmission may negatively affect the fitness of hosts.

Several other proposals also exist explaining the evolution of plant-feeding  
insects. Among those several, the slow-growth/high-mortality hypothesis (Williams,  
1999), physiological efficiency hypothesis (Dethier, 1954), and enemy-free  
hypothesis (Bernays, 1998), particularly refer to the eco-physiology of such insects.  

In the present study I have explored the relationship and dependence of the  
plant-feeding insect *E. postvittana* on *B. cinerea*-infected and uninfected leaves and  
berries of *V. vinifera*. *Botrytis cinerea* infection of *V. vinifera* is necrotrophic, which  
precludes the several contextual possibilities as outlined in the above hypotheses.  
For instance, the transmissive mother hypothesis does not apply to this specific  
context, because *E. postvittana* adult females do not transmit the conidia of *B.  
cinerea* to her offspring. Keeping the above limitations, in the present study, I have  
tested the oviposition behaviour of *E. postvittana* with respect to ‘slow-growth/high-  
mortality’, ‘optimal oviposition’ and ‘selfish motherhood’ hypotheses, because of  
their relevance and applicability.

1.3. Participants in this study

Plant-feeding insects and pathogens have a severe negative consequence to  
grapevine industry of Australia. In Australian vineyards, *E. postvittana* often  
encounter *B. cinerea* during oviposition, whereas their larvae co-occur with *B.*
cinerea feeding on their hyphae and spores (Bailey et al., 1996) establishing a three-way interaction. In the following paragraphs I will provide a contextual background for the three participants as deemed appropriate.

1.3.1. Vitis vinifera

In a global context, Australia ranks 12th in the area of vines planted, 10th in grape production, and as the fourth largest exporter (DAFT, 2008). Grape production constitutes Australia’s largest horticultural industry, covering a little more than 150,000 ha (as of 2009—2010), crushing 1.56 million tonnes of fresh grapes, and producing 1.14 billion litre of beverage wine. Australia exported more than 70 million litres wine, earning Au$ 1.88 billion in 2011 (Wine Australia, 2015). The main wine production areas are in the south-eastern region of Australia, particularly, in the states of South Australia, New South Wales, and Victoria. However, pockets of wine-growing regions in the states of Western Australia, Tasmania, and Queensland, (Wine Australia, 2015) also exist. Australia farms around 42 varieties of V. vinifera including Chardonnay, Riesling, Sauvignon Blanc, Sémillon, and Pinot Gris. Vitis vinifera is threatened by diverse plant-feeding arthropods and pathogens. Seven intensely damaging and 27 moderately damaging arthropod taxa are known exclusively from Australian vineyards (Baker et al., 1994) including species of Acarina, Hemiptera, and Lepidoptera (Bailey et al., 1994). Epiphyas postvittana and Phalaenoides glycinae (Lepidoptera: Noctuidae) are the most commonly occurring Lepidoptera that inflict the most of the damage in Australia (Bailey et al., 1994).

1.3.2. Epiphyas postvittana

Epiphyas postvittana is an insect of concern, not only in vineyards, but also in other horticultural-crop ecosystems such as apple, pear, citrus, peach, nectarine, apricot orchids, and to a lesser extent those of some vegetable and flower crops (Nicholas et al., 1994; Wearing et al., 1991). Epiphyas postvittana was first described as Teras postvittana and subsequently was treated under Tortrix, Archips, Cacoecia, and
*Eulia* by various authors. *Epiphyas* was validated by Turner (1927). Presently, *Epiphyas* includes 40 species, all known from Australia (Brown et al., 2010).

*Epiphyas postvittana* is endemic to southeast Australia, but has spread to Western Australia, Tasmania, and New Zealand (Bradley et al., 1973). It was recorded in Cornwall, England in 1937 (Meyrick 1937), but has presently spread to other parts of UK as well (Bradley et al., 1973; Bond, 1998). It was also recorded in California, USA in 2006 (Brown et al., 2010). Recent interception of *E. postvittana* in the Netherlands (Wolschrijn & Kuchlein, 2006) and Sweden (Suckling & Brockerhoff, 2010) indicates that it has the potential to become an invader in temperate and subtropical regions worldwide (Suckling & Brockerhoff, 2010). The spread of *E. postvittana* beyond its native region in southeast Australia is facilitated by its wide host range and its association with fresh fruits exported from Australia (Suckling & Brockerhoff, 2010). Lack of winter diapause and polyphagous nature has enabled *E. postvittana* to establish in new, geographically distant environments.

The life-cycle of *E. postvittana* comprises four stages, egg, larva, pupa, and adult (Figure 1.1). The forewing pattern between adult males and females of *E. postvittana* varies (females: 7–13 mm; males: 6–10 mm). Forewing colour in adult females ranges from rusty brown to pale yellow with brown–dark brown markings and that of the males is paler than those of the females, usually with a prominent median fascia (Figure 1.2d). Hindwings of both male and female are usually pale brown–grey, either uniform or with dispersed mottled with wavy dark-brown markings (Figure 1.2e). Eggs are laid in tectiform masses on the upper surfaces of plant leaves (Figure 1.2a). When newly laid, the eggs are pale yellow–white and translucent; the embryos become visible with maturation. The neonate caterpillar is usually pale yellow-green, 1.5–2 mm long, with a dark-brown head. Mature larvae (10–20 mm) are generally yellowish green (Figure 1.2b). The pupa usually lives within a membranous silky cocoon, constructed between two webbed leaves. On maturation, the pupa turns from green to brown and will be dark reddish-brown (4–5 days) and 10–15 mm long (Figure 1.2c).
This page is intentionally left blank
Figure 1.1. Life-cycle of *Epiphyas postvittana*.
This page is intentionally left blank
1.3.2.1. Economic impact

*Epiphyas postvittana*’s impact on agricultural and natural ecosystems varies in different countries. In Australia, its direct impacts include damage to various fruits and crops, seedlings of trees and ornamental plants. *Epiphyas postvittana* on *V. vinifera* can damage unmanaged vineyards as high as 70% (Wearing *et al*., 1991). The timing of *E. postvittana* infestation has a major effect on the type of damage it causes to berries. Early infestations cause immense direct losses, because the larvae feed on the stalks of berry clusters, thus destroying entire bunches. With the progress of the season, the larvae feed more on berries and the damage is restricted to fewer berries (Lo & Murrell, 2000). Bailey *et al.* (1996) estimated losses up to Au$ 2000/ha in one season. Weather conditions and variations in the populations of *E. postvittana* are key factors that affect the yield. An economic-risk analysis for *E. postvittana* in the United States of America, using a probability model for costs to major fruit crops, *V. vinifera, Malus domestica, Citrus sinensis,* and *Pyrus communis* indicates the mean annual damage is US$105 million (Fowler *et al.*, 2009).

However, the level of damage arising from feeding by the larvae of *E. postvittana* is much lesser than that arising consequently. Feeding by *E. postvittana* larvae renders the berry bunches vulnerable to attack by *B. cinerea* (Buchanan, 1977; Lo & Murrell, 2000) either due to deposition of the conidia in berry bunches or due to damaged sites on *V. vinifera* exposed to *B. cinerea* (Lo & Murrell, 2000). The conidia of *B. cinerea* usually remain caught and entwined between body segments and hairs on the larvae of *E. postvittana*, which facilitate transmission of the conidia mechanically and incidentally. Viable conidia of *B. cinerea* also occur in the frass of feeding larvae, which serve as inoculum in spreading the fungus (Bailey *et al.*, 1997).

1.3.2.2. Phenology

In Australia, *E. postvittana* usually has 3–4 generations/year depending on prevailing climate and edaphic factors (Danthanarayana, 1975; Wearing *et al*., 1991). The summer generation starts from the eggs laid during end December–early January. The larvae pupate in March–April establishing the first generation of adults
in April. The autumn–winter generation starts from eggs laid in April. These eggs hatch and overwinter as larvae and pupate in September. The overwintering larvae occur in small numbers in June–September. These larvae do not undergo diapause. Adults emerging in September–October lay eggs for spring generation in October, which grow into adults in December establishing the summer generation. During warm months (December—March), an overlap of spring and summer generations can occur (Danthanarayana, 1975). In laboratory trials, the minimum temperature required for the development is estimated at 7.5°C and the rate of development increases linearly with increasing temperature until about 28°C, beyond which the rate of growth declines (Gu & Danthanarayana, 1992). Egg hatch and larval development cease above 30°C. Wind speed is one key factor for active flights of *E. postvittana*. The lower and upper thresholds of the wind speed for the flight of *E. postvittana* is 0.5 m/sec and 2.6 m/sec (Danthanarayana, 1976). Humidity plays no critical impact on the males of *E. postvittana*, whereas the female moths respond less favourably at atmospheric humidity <9.6%, and the maximum flight duration occurs at a 55.7% atmospheric humidity (Gu & Danthanarayana, 1992).

1.3.2.3. Population dynamics

In 24 h after emergence, females mate, and eggs are laid in tectiform clusters (~30 eggs) (see Figure 1.2a). A female lays 150–1500 eggs in optimal laboratory conditions. In contrast only 100–300 eggs are laid in natural conditions, influenced by atmospheric temperature and food plant quality (Danthanarayana, 1983; Suckling & Brockerhoff, 2011).

Neonate larvae emerge after 1–2 weeks of oviposition (Suckling & Brockerhoff, 2011). Once established, the larvae feed from within rolled leaves and remain sedentary. Larvae (see Figure 1.2b) have five (males) or six (females) instars and involve 25–40 d to go through developmental stages, when optimum temperature (20–25°C) prevails. Larval development also depends on the plant species on which larvae feed. On maturation the pupae (see Figure 1.2c) turn from green to brown and the males (see Figure 1.2d) emerge before the females (see
Figure 1.2e). The damage causing stage of *E. postvittana* is the larva, which feeds on buds, foliage, shoots and fruits (Suckling *et al*., 1998; Markwick *et al*., 2003).

1.3.2.4. *Behaviour of the larvae*

As neonate larvae emerge, they search for a settlement site and may disperse via ballooning silk thread, depending on weather conditions. *Epiphyas postvittana* larvae are thigmotactic. Rolling and webbing of leaves to create shelters can result in damages mainly to leaves and occasionally to fruits (Suckling & Brockerhoff, 2011; Lo *et al*., 2000). Shelter construction is not only a protective tactic against parasitoids but also it serves a variety of functions such as preventing dislodgement and desiccation (Loeffler, 1996; Larsson *et al*., 1997). At the time of emergence, the larvae are mobile and can move to other plant parts. In terms of preference between host and non-host species, the larvae of *E. postvittana* distinguish and feed on the host plants rather than on nonhosts. The larvae may abandon the site if the food quality is inappropriate (Harris *et al*., 1997). Chewing by the larvae of *E. postvittana* on the leaves and berries of *V. vinifera* inflicts injuries, which render them vulnerable to various diseases including the grey mould disease caused by *B. cinerea*. In Australia, *véraison* (onset of ripening) occurs in late January on varieties of *V. vinifera*, so the infestations by *E. postvittana* larvae in February and March often coincide with the emergence of berries, thus rendering them highly vulnerable to infection by *B. cinerea*.

1.3.2.5. *Host range*

The host range of *E. postvittana* is exceptionally wide, with Australian records indicating 123 generic taxa placed in 55 families (Geier & Briese, 1980; Danthanarayana, 1975). The potential list of host plants for *E. postvittana* mostly includes dicotyledonous plants, although a few monocotyledons, conifers, and even ferns are infested (Brockerhoff *et al*., 2011). The prevalence of Rosales, Fabales, Vitales and Saxifragales could possibly be related to observers’ bias toward economically important plants.
1.3.2.6. Host selection

In *E. postvittana* host selection is influenced by plant volatiles (Suckling *et al.*, 1996) and surface cues (Foster & Howard, 1998), because of vision, chemoreception, and olfactory receptors. Attraction of *E. postvittana* females to port-wine bait traps and pheromone traps in the field (Suckling *et al.*, 1994) indicates that orientation to volatile compounds over long-distance is a strong possibility. Certain plant odours arising from different alcohols, ketones, aldehydes, esters, and terpenes are electro-physiologically active and mediate oviposition in *E. postvittana* (Suckling *et al.*, 1996). For example, citral inhibits attraction of gravid females and deters oviposition (Suckling *et al.*, 1996). On the other hand, eugenol and geraniol do not play a role in attraction but inhibit oviposition (Suckling *et al.*, 1996). At least three odour receptors (identified as ‘EpOR1’, ‘EpOR2’, and ‘EpOR3’) have been identified in the antennae of *E. postvittana* that recognize volatiles, particularly methyl salicylate (Jordan *et al.*, 2009).

Tactile stimuli also play a role in host selection for oviposition. *Epiphyas postvittana* is greatly influenced by surface stimuli including surface texture-related stimulus (Roessingh & Städler, 1990; Foster *et al.*, 1997). Females of *E. postvittana* prefer to lay eggs on smooth surfaces (Danthanarayana, 1975; Foster *et al.*, 1997) with varicose texture (Foster & Howard, 1998), rather than on either rough or hairy surface (Tomkins *et al.*, 1991; Foster *et al.*, 1997).

1.3.2.7. Host susceptibility

*Epiphyas postvittana* lives on several fruit crops, including *V. vinifera* in Australasia. Damage caused by *E. postvittana* to *V. vinifera* costs Australia up to Au$ 2,000/ha pa (Bailey *et al.*, 1995). *Epiphyas postvittana* can complete its life cycle, feeding on leaves and berries of *V. vinifera* (Mo *et al.*, 2006). One *E. postvittana* female can lay up to 1500 eggs, although atmospheric temperature and the food consumed during larval stages are strong factors that influence the egg numbers (Danthanarayana, 1983).
Figure 1.2 (A) egg mass, (B) larva, (C) pupa on leaf surface,(D) adult female, (E) adult male of *Epiphyas postvittana* (Photos by Syed Rizvi).

(bar=1mm)
This page is intentionally left blank
In Australia, *E. postvittana* infests certain varieties such as, Chardonnay, Sémillon, and Sauvignon Blanc, more intensely than the others, such as, Shiraz, Cabernet Sauvignon, and Merlot (Paull, 2008; Weeks & Pitman, 2012). Differential susceptibility of the varieties Chardonnay and Cabernet Sauvignon to *E. postvittana* has been documented, with differences attributable to either to either in larval settlement or to oviposition preference patterns (Paull, 2008). *Epiphyas postvittana* shows flexibility in its behavioural, physiological, and demographic performances, while responding to environmental heterogeneity (Gu & Danthanarayana, 2000). Considerable differences in life-history traits and population-growth parameters within and among populations have been shown (Gu & Danthanarayana, 2000).

1.3.2.8. Management

To manage *E. postvittana* populations in Australia, DDT (dichloro-diphenyl-trichloroethane) was introduced in the 1950s (Thwaite *et al*., 1993). Azinphos-methyl was also used in later years, however resistance to it developed in *E. postvittana* in the next two decades (Suckling *et al*., 1984). *Bacillus thuringiensis kurstaki* (Bacillales: Bacillaceae) is effective in regulating *E. postvittana* populations in recent years (Stevens & McKenna, 1999).

Egg parasitoids such as *Trichogramma carverae* (Hymenoptera: Trichogrammatidae), *Xanthopimpla rhopaloceros* (Hymenoptera: Ichneumonidae), *Glabridorsum stokesii* (Hymenoptera: Ichneumonidae), and *Trigonospila brevifacies* (Diptera: Tachinidae) are currently used as a biocontrol agents against *E. postvittana* infestation (Suckling *et al*., 1984).

1.3.3. *Botrytis cinerea*

*Botrytis cinerea* Pers. (1794) (Helotiales: Sclerotiniaceae) is the causal agent for the ‘grey mould’ disease developing on >200 plant species, such as *Brassica oleracea* (Brassicaceae), *Lactuca sativa* (Asteraceae), *Phaseolus vulgaris* (Fabaceae) and fruit crops (*Fragaria ananassa*, *Rubus occidentalis*, *Rubus fruticosus*, and *V. vinifera*) (Williamson *et al*., 2007). *Botrytis cinerea* is a necrotroph: intense infection results
in the death of host tissues and *B. cinerea* can survive and sporulate as saprophytes on dead tissues and can produce long-term survival structures, such as sclerotia (Elad *et al.*, 2007). The sclerotia remain associated with both living plant parts and plant debris on the soil.

1.3.3.1 **Biology of Botrytis cinerea**

*Botrytis* comprises a little more than 20 species. Phylogenetic analysis of 22 species of *Botrytis* revealed that *B. cinerea* forms a small clade with three other species, all of which are specialized pathogens of dicotyledons (Staats *et al*., 2005).

The life cycle of *Botrytis cinerea* comprises a vegetative, mycelial system that produces asexual macroconidia, sclerotia and microconidia. Sclerotia germinate to produce the mycelium and the conidia, but after appropriate preconditioning and fertilization, they produce the apothecia containing ascospores. Mycelia, sclerotia and conidia have different abilities for survival and dispersal, and the relative roles of these structures will vary greatly depending on the crop ecosystem and seasons. Both hyphal cells and conidia are multinucleate (3—6) (Shirane *et al*., 1988). Microconidia are, on the other hand, uninucleate, and seldom germinate on laboratory media, and they apparently function primarily as male gametes in sexual crosses. (Faretra & Antonacci, 1987).

1.3.3.2 **Geographical incidence and host range**

In general *B. cinerea* occurs wherever their hosts are cultivated as crops, ranging from warm (tropical and subtropical) to cold (temperate) areas (Have *et al*., 1998; Williamson *et al*., 2007). Usually conidial germination, mycelial growth and production of conidia occur rapidly on potential host plants. In effect, *B. cinerea* results in high levels of production loss, especially because a majority of the host plants are economically important (Williamson *et al*., 2007). In field conditions *B. cinerea* is active at 15–25°C. However, its ability to be active even at 0°C makes it a significant pathogen particularly of stored fruits (Thomas *et al*., 1988; Steel *et al*.,
2011). *Botrytis cinerea* sporulates profusely and dry conidia are wind dispersed, thus making this pathogen a constant threat to susceptible crops.

1.3.3.3. *Interaction with grapes*

As a pathogen of *V. vinifera*, vineyards managers consider *B. cinerea* a significant threat (Vivier & Pretorius, 2002) because of the highly estimated crop losses due to this pathogen and estimated crop losses (US$ 2 billion/annum). According to Kable (1991), an epidemic caused by *B. cinerea* in New South Wales alone, in 1986–1987 growing season resulted in 10% crop loss, while in 1989–1990, loss from *B. cinerea* induced damage spiked to 20%. In a vineyard, several sources of inocula of *B. cinerea* occur, including viable sclerotia, pruned vine stems and rootstocks, and necrosed *V. vinifera* tissues on both the vine and the soil (Elad et al., 2007). Release of viable conidia from these sources in favourable seasons provides for an abundant inoculum that can infect berries (Figure 1.3) and young shoots and leaves of *V. vinifera* (Nair et al., 1995). *Botrytis cinerea* has the potential to completely destroy *V. vinifera* berries (Figure 1.4). Alternatively, under certain conditions, it may cause a slow decay permitting the berries to desiccate. Temperature significantly influences growth of *B. cinerea. Botrytis cinerea* can survive at 0°C with low mycelium development, but can grow well at 20°C (Steel et al., 2011); 30°C is the upper threshold limit for the survival of *B. cinerea* (Thomas et al., 1988). Wind speed, relative humidity, and temperature are limiting factors in the development of mycelium by *B. cinerea* on *V. vinifera*. The greatest number of conidia is usually produced at 16–21°C at 94% (RH), and 0.6 m/sec wind speed (Thomas et al., 1988).
Figure 1.3 Conidiophore with conidia of *Botrytis cinerea* from *V. vinifera* berries (Photo by Syed Rizvi). (Bar=10 μm)

Figure 1.4 Berries of *V. vinifera* infected by *B. cinerea* (Photo by Syed Rizvi).
This page is intentionally left blank
2. THE PURPOSE OF THIS STUDY AND RESEARCH QUESTIONS

In this thesis, I hypothesize that Chardonnay is the most susceptible variety of *V. vinifera* for *E. postvittana'*s infestation and infection of *V. vinifera* by *B. cinerea* influences the bionomics of *E. postvittana*. Keeping the above in view, I will be presenting the life-history performance of *E. postvittana* on varieties Chardonnay, Sauvignon Blanc, Merlot, Marsanne, and Semillon of *V. vinifera*; the oviposition, olfactory behaviour, and life-history performance of *E. postvittana* on *B. cinerea*-infected and uninfected leaves and berries of *V. vinifera* cv. Chardonnay; the volatile composition of uninfected and *B. cinerea*-infected leaves and berries of *V. vinifera*, and profiling sterols in leaves and berries of *V. vinifera*, the mycelia of *B. cinerea*, and the larvae that were fed on these materials.

The research questions, therefore, addressed are:

- Do the varietal differences in *V. vinifera* influence the life-history performance of *E. postvittana*?
- Do the infection of *V. vinifera* with *B. cinerea* influence the bionomics of *E. postvittana*?
  - Do *B. cinerea* infected leaves of *V. vinifera* influence the preference pattern of *E. postvittana*?
  - Do *B. cinerea*-induced change in *V. vinifera* leaves influence the selection behaviour and life history of *E. postvittana*?
  - Does *B. cinerea* material incorporated with synthetic nutritional media affect the development of *E. postvittana*?
  - Do *B. cinerea*-induced changes in *V. vinifera* berries influence the oviposition behaviour and life history of *E. postvittana*?
  - Does *B. cinerea* alter the volatile composition of *V. vinifera* berries and influence the selection behaviour of *E. postvittana*?
3. REFERENCES


Whitehill, J.G., Lehman, J.S. and Bonello, P. (2007) Ips pini (Curculionidae: Scolytinae) is a vector of the fungal pathogen, Sphaeropsis sapinea


Chapter 2

Variations in the chemistry in the leaves of five varieties of *Vitis vinifera* and their implications in the performance and development of *Epiphyas postvittana*.

*Published paper (details below) presented with minor modifications.

This page is intentionally left blank
1. INTRODUCTION

Foliar chemistry (relative composition of nutrients and secondary metabolic compounds) can modify the performance of plant-feeding insects in diverse ways (Mattson, 1980; Awmack & Leather, 2002). Nitrogen content of a plant is critical for the growth and development of plant-feeding insects and strongly influences their feeding behaviour (Mattson, 1980). For example, the consumption rate of the larvae of *Samea multiplicalis* (Lepidoptera: Pyralidae) is greater when feeding on *Salvinia molesta* (Salviniaceae) with a low nitrogen content, relative to *S. molesta* with high nitrogen contents (Taylor, 1989). On the other hand, *Acyrthosiphon pisum* (Hemiptera: Aphididae) feed more-or-less continuously on plant-phloem sap, which is high in sucrose concentration (Abisgold *et al*., 1994). Carbohydrates have a major impact on the performance of plant-feeding insects, but high levels of sugars in plant tissues often have negative effects on insect development, because these sugars influence the quality of other nutrients, thus requiring the insects to increase their consumption rate to offset deficiencies (Vanderzant & Richardson, 1963; Bartlet *et al*., 1990). When individuals of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) were reared on a synthetic diet, their population growth rates were negatively influenced by concentrations of sucrose in their diet (Clancy, 1992). In contrast to nutrients, the impacts of secondary compounds of the host plant are usually negative in terms of their influence on larval and adult performance of plant-feeding insects (Vanderzant, 1973; Awmack & Leather, 2002). When *Trichoplusia ni* (Lepidoptera: Noctuidae) was reared on cellulose-based diets, survival rate was 88%. However, when secondary plant compounds of *Solidago altissima* (Asteraceae) were added to the diet, mortality was nearly 100% (Bosio *et al*., 1990). Collectively, the effects of foliar chemistry on feeding behaviour have implications on the nutritional physiology of plant-feeding insect, and through that, influence their life-history traits (Dadd, 1985; Danthanarayana *et al*., 1995; Moreau *et al*., 2006).

Life-history traits of the Tortricidae vary with variations in host quality (Danthanarayana *et al*., 1995; Moreau *et al*., 2006). Life-history performance involving longer-development time, lower-pupal mass, higher-mortality rate, and greater numbers of instars reinforce the suboptimal quality of the host (Frago & Bauce, 2014). Nutritional quality varies between plant species and also among
varieties (Thiéry & Moreau, 2005; Moreau et al., 2006). Such a variation determines larval and adult performances among the Lepidoptera, which require stored energy for reproductive performance. For instance, oocyte production in Lepidoptera depends on the quality of food consumed during larval periods (Boggs, 1997). In consequence, density of insect populations generally correlates positively with the food quality. For example, *L. botrana*, when reared on the berries of *V. vinifera* (grapevine) varieties, *viz.*, Chardonnay, Chasselas, Gewurztraminer, Grenache, and Lambrusque, their larval-development time, fecundity, egg size, and the proportion of larval hatching vary significantly (Moreau et al., 2006).

*Epiphyas postvittana* lives on several fruit crops, including *V. vinifera* in Australasia. Damage caused by *E. postvittana* to *V. vinifera* costs Australia up to Au$ 2,000/ha pa (Bailey et al., 1995). *Epiphyas postvittana* has invaded many regions in the world (Suckling & Brockerhoff, 2010). *Epiphyas postvittana* can complete its life cycle, feeding on leaves and berries of *V. vinifera* (Rizvi & Raman 2015a; Rizvi et al., 2015). One *E. postvittana* female can lay up to 1500 eggs, although atmospheric temperature and the food consumed during larval stages are strong factors that influence the egg numbers (Danthanarayana, 1983).

In Australia, *E. postvittana* infests certain varieties such as, Chardonnay, Sémillon, and Sauvignon Blanc, more intensely than the others, such as, Shiraz, Cabernet Sauvignon, and Merlot (Paull, 2008; Weeks & Pitman, 2012; Baker, 2005). Differential susceptibility of the varieties Chardonnay and Cabernet Sauvignon to *E. postvittana* has been documented, with differences attributable to either to either in larval settlement or to oviposition preference patterns (Paull, 2008). *Epiphyas postvittana* shows flexibility in its behavioural, physiological, and demographic performances, while responding to environmental heterogeneity (Gu & Danthanarayana, 2000). Considerable differences in life-history traits and population-growth parameters within and among populations have been shown (Gu & Danthanarayana, 2000).

Keeping the above in mind, I reared *E. postvittana* on lyophilized leaf material of Chardonnay, Marsanne, Sauvignon Blanc, Merlot, and Sémillon incorporated individually into an amended artificial diet (Rizvi & Raman, 2015b)
maintained under laboratory conditions. I tested how varieties can influence the life-history performance of the larvae and the female-reproductive performance of *E. postvittana*. The variety Marsanne was included in this study to explore why *E. postvittana* rarely occurs on Marsanne. [Inclusion of Marsanne in this study was also because many of the vineyard managers in central-west New South Wales believe that Chardonnay is the most susceptible to *E. postvittana* and Marsanne is the least.] The larvae were also reared on the synthetic-nutrient diet with no *V. vinifera* leaf material added to it. I sought answers to the following questions: (1) Does foliar chemistry differ among the selected varieties of *V. vinifera*? (2) Does varietal difference influence the life-history performance of *E. postvittana*? To verify these questions, I determined total nitrogen, carbohydrates, phenols, and nutrients (potassium, iron, sodium, and zinc) from the five selected varieties of *V. vinifera*. The performance of *E. postvittana* was examined by measuring its life-history traits from egg hatch to death, evaluating *E. postvittana* fitness reared on the selected varieties. Pupal mass, larval-development time, and larval-survival rate were recorded, and the influence of plant quality on reproductive success was determined, by counting the number of eggs laid, percentage of egg hatchability, and adult longevity.

2. MATERIALS AND METHODS

2.1. Insect culture

Eggs of *E. postvittana* were obtained from pure-laboratory cultures of insects maintained at the School of Agriculture, Food, and Wine, Waite Campus, The University of Adelaide, Adelaide. The neonate larvae were maintained on semi-synthetic diet in plastic containers (35x20x4 cm³). The semi-synthetic diet contained 250 g dry lima beans [*Phaseolus lunatus*], 1600 ml water, 32 g agar, 8 g ascorbic acid, 80 g dried Brewer’s yeast, and 5 g paraben. The diet was autoclaved (Tomy, ES–315, Tokyo, Japan) at 121°C for 20 min and cooled to 65–70 °C before adding 8.0 g ascorbic acid and 4 ml formaldehyde. The diet was blended in a blender. This hot diet was poured to depths of 2 cm in rearing plastic containers. Three to five individual egg masses were cut from the corrugate-walled Dixie cup (for details, see
below) and inserted into the diet layer in the rearing containers. The larvae were maintained in incubator (Sanyo Incubator, MIR–253, Osaka, Japan) at 21±1°C, 60–80% RH, and 16L: 8D regimen. On pupation, the insects were sexed and segregated considering total-body length and numbers of abdominal segments. A 24-h old adult female was paired with a similar aged adult male in a corrugated-walled Dixie cup. These pairs were fed on cotton balls soaked in 10% aqueous-honey and 0.1% sorbic acid. Oviposition occurred on the walls of cups, which were later cut into rafts bearing egg masses with numbers varying from 3 to 95. Some of the rafts were stored at 4–6°C for future use, and the remaining was used to raise adults. The eggs (5–6 d old) were surface-sterilized using 5% formaldehyde solution and then washed with sterile water (Bürgi & Mills, 2010). A 24 hour-old female was isolated with a similar-aged male in the Dixie cup. After 24 h, only those females that laid about 10 eggs were used in the experiments, the details of which are provided in the following sections. Adult females were not exposed to either uninfected (control) or B. cinerea-infected V. vinifera, prior to experiments.

2.2. Lyophilization of leaf tissues of different varieties of Vitis vinifera

Leaves of V. vinifera varieties Chardonnay, Sauvignon Blanc, Merlot, Marsanne, and Sémillon (phenological stages 62–68, Lorenz et al., 1994) were collected from organic vineyard in Central-western Highlands Bioregion, NSW. Under field conditions, the larvae of E. postvittana selectively feed only on intervenal tissue. Therefore, petioles and recognizable veins were removed from the leaves and the intervenal tissues were washed several times with distilled water and lyophilized using a lyophilizer (Heto DryWinner, CT/DW 60E, Allerød, Denmark). The lyophilized leaf materials were ground in a motorized grinder (Glen Creston Ltd, Stanmore, Middlesex, UK) and stored at −80 °C. I used lyophilized leaf material since it enabled us to control environmental and nutritional variables. Moreover, use of lyophilized leaf material prevents the incidences of microbial infection and also reduces the chances of food deprivation to the larvae (Thiéry & Moreau, 2005; Moreau et al., 2006).
2.3. Leaf chemistry

Lyophilized leaf materials of each variety were used to determine total nitrogen, carbohydrates, phenols, and nutrients. Total nitrogen was determined using Kjeldahl technique (Association of Official Analytical Chemists, 2012) with 0.5 g of the selected lyophilized leaf material. Total-carbohydrate contents were determined via colorimetric assay using Anthrone reagent (Sigma-Aldrich, St. Louis, MO) (Quarmby & Allen, 1989) using 0.03 g of the selected lyophilized leaf material. Total phenols were measured following Qawasmeh et al. (2012) using 2.0 g of the selected lyophilized leaf material. The nutrients (cations) were measured using 0.5 g of the selected lyophilized leaf material in a Perkin-Elmer 200 Atomic-Absorption Spectrophotometer equipped with a hollow cathode lamp, a deuterium background corrector, and an air–acetylene (10:2.5) flame. The instrument parameters applied here were as recommended by Perkin-Elmer (Perkin-Elmer, 2006).

Sodium, iron, potassium and zinc are essential for normal growth and development of Lepidoptera (Hasegawa & Ito, 1967; Malik & Malik, 2009). In the haemolymph of phytophagous lepidopteran larvae, the cation composition is characterized by high potassium and low sodium concentration (Jungreis, 1978). Therefore, Na, Fe, K, and Zn were chosen as representative elements in this section of the investigation.

2.4. Synthetic diets for the larvae

The synthetic diet for the larvae of *E. postvittana* was developed using the diet formula of Thiéry and Moreau (2005) with minor modifications. In this diet, in the present experiment, linoleic acid was used instead of maize oil. Maize oil is a key source for linoleic acid, an essential fatty acid for insects, particularly the Lepidoptera (Canavoso *et al.*, 2001; Duckett *et al.*, 2002). Maize oil also includes oleic acid (Duckett *et al.*, 2002), which does not favour growth in the Lepidoptera (House & Barlow, 1960). In this diet, I replaced benzoic acid, used as an antifungal agent (Cohen, 2003) by Thiéry and Moreau (2005), with 95% ethanol, since benzoic acid addition would not enable us to bring the pH to 6.5. Singh (1974) indicates that
6.5 is the most appropriate pH in the synthetic diet for *E. postvittana*. The pH was adjusted to 6.5 using 4N KOH following Singh (1974).

Agar, casein, cellulose powder, and Wesson’s salt mixture were mixed and a mixture of linoleic acid, cholesterol, and dichloromethane was added. The diet was left in fume cupboard overnight to allow the solvent to evaporate. Distilled water and KOH were added to the diet and autoclaved at 121°C for 20 min. The diets were cooled to 50–55°C before adding Vanderzant vitamin mixture, glucose and streptomycin. Sorbic acid, paraben, and 95% ethanol were mixed in a separate beaker and added with diet. This constituted the basic diet. Lyophilized leaf material of different varieties of *V. vinifera* (7.8 g each) was added individually in separate containers and mixed with the basic diet. Each diet was distributed equally into three plastic food containers (20x15x7 cm³, referred as ‘containers’ hereafter) and allowed to cool in a laminar air flow cabinet (Gelaire®, AHS-104-C, Sydney, Australia) for 2 h. The basic diet, with no leaf extract included, served as the control. The containers including the amended diet with added lyophilized leaf material of different varieties of *V. vinifera* will be referred as ‘amended diet+Chardonnay’, ‘amended diet+Merlot’, and so on (Table 2.1).

2.5. **Life history performance of *Epiphyas postvittana***

2.5.1. *Growth and development*

Three batches of 30 neonate larvae (*n*=90) were distributed in three containers that included either the control or the amended diet. Use of 20x15x7 cm³ containers was necessary to minimize competition among the 30 introduced larvae in each of them. The larvae were monitored every 24 h until pupation. On pupation they were removed from the containers and each pupa was weighed (A&D Weighing Scale, HM–200, Massachusetts, US0029). The measured pupal mass was used as an index of adult-body mass, because adult body mass could only be obtained destructively. Each pupa was quarantined to a glass vial (4 cm tall, 2 cm diameter) closed with a ventilated plastic stopper and stored in the incubator at 22°C until emergence. Adults were sexed on emergence.
Total survival rate (= adult emergence rate %), total development time (from the neonate larva to pupation, from pupa to adult), mass of pupae, and adult sex ratio were measured.

2.5.2. Adult performance

Adults were paired by placing a 24 h-old female and a similar-aged male in a corrugate Dixie™ cup (200 ml) covered with Parafilm®. A water-soaked cotton wick was suspended into the cup through the Parafilm (see Rizvi & Raman, 2015b). Feeding adults on water was necessary to achieve maximum fecundity (Gu & Danthanarayana, 1990). Adult males and females used in these trials were randomly selected from those reared in the laboratory. Adults were confined to Dixie cups until death. Eggs were incubated at 22°C, 16L: 8D regimen for 15 d to measure the fertility rate. Adult performance was measured using the fecundity rate, possible delay in oviposition after emergence from pupae (measured in d), and fertility of females (% of hatched eggs). The number of days from the date of emergence of adults from pupae to death was also recorded.

2.5.3. Female-fitness index

Female-fitness index was calculated using the following equations:
(i) Number of emerged larvae x survival rate = number of adults emerged [A].
(ii) A x female sex ratio = number of females contributing to the next generation [B].
(iii) B x mating success = female-fitness index.
Table 2.1 The composition of the control diet and *V. vinifera*-enriched diet

<table>
<thead>
<tr>
<th>Materials</th>
<th>Control</th>
<th><em>V. vinifera</em>-enriched diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (ml)</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Agar (g)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Casein (g)</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Wesson salt mixture (g)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cellulose (g)</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Vanderzant vitamin mixture (g)</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Linoleic acid (ml)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Cholesterol (g)</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>4N KOH (ml)</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Sorbic acid (g)</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Paraben (g)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>95% Ethanol (ml)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptomycin (g)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Freeze-dried powder of leaves of <em>V. vinifera</em> (g)</td>
<td>—</td>
<td>7.8</td>
</tr>
</tbody>
</table>
2.5.4. Discriminant analysis

Larval-development time, pupal mass, fecundity, and adult life span were used to develop a discriminant model for representing the life traits of *E. postvittana* reared on control and amended diets. The representation of varieties of *V. vinifera* is defined in the first two canonical functions. A stepwise discriminant analysis was applied using a ‘forward’ procedure.

2.6. Statistical analysis

Prior to statistical analysis, the data from each experiment were checked for normality, applying Shapiro–Wilk’s Test. Kruskal–Wallis and Wilcoxon Rank-Sum non-parametric tests were applied, when the data did not meet normality. A contingency table ($\chi^2$ test) followed by a Ryan multi-comparison test was applied to screen significant differences in sex ratio, percentage of pupation, and percentage of adult emergence. Hatching proportions were predicted and analyzed using a generalized-linear model (binomial distribution). A stepwise discriminant analysis was carried out using a ‘forward’ procedure, which begins with no variables in the model and adds the variables with the greatest discriminating power (Moreau *et al.*, 2006). Statistical comparisons of the data of foliar chemistry were performed using one-way ANOVA and post-hoc Bonferroni separation test. Comparison of treatment effects among varieties were considered statistically significant when $p<0.05$. Analyses were made using JMP® software (Version 10.0.0, SAS Institute Inc. 2012, North Carolina, USA) and GenStat (VSN International 2012, Hertfordshire, UK). Graphs and tables were generated in MS Excel (2013).

3. RESULTS

3.1. Leaf chemistry

Lyophilized leaf materials of Chardonnay showed significantly higher nitrogen content ($p<0.05$) than Marsanne and Sémillon (Table 2.2). I found the greatest levels of total carbohydrates in Marsanne, but the differences, when compared with other
varieties tested were not significant. Total phenols were the highest in Chardonnay and were the least in Merlot, but the differences when compared with other varieties were not significant (Table 2.2). I did not find any significant difference in nutrients values among the five selected varieties of *V. vinifera* (Table 2.3).

### 3.2. Life history performance of *Epiphyas postvittana*

#### 3.2.1. Growth and development

The proportion of larvae that pupated from the five varieties was not significantly different (80–88%), but survival of larvae fed on control diet was significantly lower (68.5%, *p*= 0.02, Table 2.4) than the larvae fed on the each of amended diets with lyophilized leaf materials from the five varieties. Male pupae were similar in mass across all diets, while female-pupal mass significantly varied among those reared on amended diets. The larvae fed on amended diet+Chardonnay achieved the greatest mass, which was significantly higher than the pupal mass of larvae fed on amended diets, except the amended diet+ Sauvignon Blanc (Table 2.4). Mass of the female pupae was greater than that of the male pupae in general. Male *E. postvittana* larvae pupated quicker than the females by 3.0±0.41 d, and therefore emerging earlier than the females on the amended and control diets, whereas the pupal duration of both males and females did not vary on any diet (Table 2.4).

#### 3.2.2. Adult performance

Female-fitness was calculated from the number of females that produced viable eggs (80–92%). Adult-male duration of *E. postvittana* fed on amended diet+Chardonnay was significantly higher than those fed on amended diet+Marsanne (Table 2.5). No significant effect of diets on the sex ratio of emerging adults occurred (Table 2.5). The time delay in oviposition was unaffected among insects reared on amended and control diets. Fecundity was significantly affected when reared on amended and control diets (Kruskal-Wallis Test, $\chi^2_{5}=19.8$, *p*=0.0014, Figure 2.1). *Epiphyas postvittana* larvae fed on amended diet+Chardonnay laid significantly higher number of eggs than those reared on amended diet+Marsanne. Among the five tested
varieties, the larval emergence was significantly greater in amended diet+Chardonnay than those reared on amended diet+Marsanne and amended diet+Sémillon (Kruskal-Wallis Test, $\chi^2 = 16.6, p = 0.005$, Figure 2.2), but not from those reared on amended diet+Sauvignon Blanc and amended diet+Merlot. The hatching proportion remained unchanged among the amended and control diets (Generalized-linear model, $\chi^2 = 0.48, p = 0.793$).

3.2.3. Female-fitness index
The fitness of females reared on control diet was the lowest compared with those reared on amended diets. The females reared on amended diet+Chardonnay showed significantly greater fitness index than those reared on other diets, except the amended diet+Sauvignon Blanc, whereas the amended diet+Marsanne or amended diet+Merlot resulted the lowest-fitness index (Kruskal-Wallis Test, $\chi^2 = 23.3, p = 0.0003$, Table 2.6).

3.2.4. Discriminant analysis
In the discriminant model, Figure 2.3 shows in term of life-history performance traits of *E. postvittana* that the control diet is separated from the amended diets. Among the tested varieties of *V. vinifera*, the amended diet+Marsanne is separated from the other varieties of *V. vinifera*. The amended diet+Chardonnay, +Sauvignon Blanc, +Merlot, and +Sémillon overlap each other indicating that *E. postvittana* reared on these diets exhibited similar life-history performance traits.

4. DISCUSSION
In this study, chemical variations in the leaves of five chosen varieties of *V. vinifera*, viz., Chardonnay, Sauvignon Blanc, Merlot, Marsanne, and Sémillon were determined, assaying total nitrogen, total carbohydrates, total phenols, and nutrients. The effect of diets amended with leaf materials of these varieties on the development and reproductive traits of *E. postvittana* was also measured. I found that the nitrogen content differs significantly among the tested varieties. Larval performance and
adult-reproductive potential of *E. postvittana* also differed significantly. The larval-development time, female-pupal mass, fecundity, and larval emergence varied significantly, whereas the larval mortality, sex ratio, pupal duration, adult life span, and hatching proportion did not. *E. postvittana* larvae reared on Chardonnay, Sauvignon Blanc, Merlot and Sémillon exhibited a positive effect in their life-history traits, whereas those on Marsanne showed poor larval development and adult performance. The larvae reared on Chardonnay developed quicker than those reared on diets including leaf materials of other varieties, attained greater pupal mass, and performed well as adults (measured as rates of fecundity and fertility). On the other hand, feeding on Marsanne resulted in slow larval development and poor adult performance with those reared on amended diets including leaf materials of other varieties.
Table 2.2 Foliar chemistry of five varieties of *V. vinifera*

<table>
<thead>
<tr>
<th>Varieties of <em>V. vinifera</em></th>
<th>Nitrogen (%)</th>
<th>Total Carbohydrates (%)</th>
<th>Total phenols&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chardonnay</td>
<td>16.1±0.39AC</td>
<td>17.2±0.15</td>
<td>9.4±0.4</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>15.3±0.58AB</td>
<td>16.3±0.39</td>
<td>7.7±1.4</td>
</tr>
<tr>
<td>Merlot</td>
<td>15.9±0.72A</td>
<td>17.9±0.35</td>
<td>7.1±0.2</td>
</tr>
<tr>
<td>Marsanne</td>
<td>12.8±0.4B</td>
<td>21.3±0.85</td>
<td>8.9±0.4</td>
</tr>
<tr>
<td>Sémillon</td>
<td>13.6±0.93BC</td>
<td>16.4±0.49</td>
<td>8.6±0.3</td>
</tr>
<tr>
<td>Statistic</td>
<td>0.002</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td><em>p</em></td>
<td>9.03</td>
<td>4.22</td>
<td>3.79</td>
</tr>
</tbody>
</table>

Values (mean±s.e) in each column with the same letter are not significantly different (p>0.05)
<sup>a</sup> = mg of gallic acid equivalents/g dry mass

Table 2.3 Potassium, iron, sodium, and zinc values (mean±s.e) of five selected varieties of *V. Vinifera*

<table>
<thead>
<tr>
<th>Varieties of <em>V. vinifera</em></th>
<th>Potassium (ppm)</th>
<th>Iron (ppm)</th>
<th>Sodium (ppm)</th>
<th>Zinc (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chardonnay</td>
<td>14899.8±447.9</td>
<td>85.2±16.9</td>
<td>288.5±10.5</td>
<td>46.9</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>15567.1±310.7</td>
<td>97.8±5.4</td>
<td>309.0±2.5</td>
<td>41.7</td>
</tr>
<tr>
<td>Merlot</td>
<td>15411.9±216.46</td>
<td>74.1±4.1</td>
<td>277.1±2.9</td>
<td>39.5</td>
</tr>
<tr>
<td>Marsanne</td>
<td>14125.9±33.39</td>
<td>60.4±2.1</td>
<td>308.4±6.0</td>
<td>49.8</td>
</tr>
<tr>
<td>Sémillon</td>
<td>14471.3±348.1</td>
<td>93.1±1.3</td>
<td>283.4±10.36</td>
<td>38.6</td>
</tr>
<tr>
<td>Statistic</td>
<td>5.09</td>
<td>3.32</td>
<td>4.04</td>
<td>3.87</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.05</td>
<td>0.11</td>
<td>0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Table 2.4 Surviving from hatching to pupa, larval developmental time, pupal duration, pupal mass, and Surviving from pupa to adult of *E. postvittana* reared on different varieties of *V. vinifera*.

<table>
<thead>
<tr>
<th>Larval Food</th>
<th>Larval survival (surviving from hatching to pupa) (%)</th>
<th>Larval developmental time (day)</th>
<th>Pupal duration (days)</th>
<th>Pupal mass (mg)</th>
<th>Adult emergence (surviving from pupa to adult) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Control</td>
<td>68.5A</td>
<td>32.7±0.7A</td>
<td>33.8±0.6A</td>
<td>10.2±0.2</td>
<td>9.4±0.2</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>88.7B</td>
<td>28.2±0.8B</td>
<td>29.7±0.6B</td>
<td>9.7±0.2</td>
<td>9.5±0.1</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>86.6B</td>
<td>28.8±0.8B</td>
<td>31.7±0.6CD</td>
<td>9.9±0.1</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>Merlot</td>
<td>83.3B</td>
<td>27.9±0.3B</td>
<td>30.2±0.6BD</td>
<td>10.3±0.1</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>Marsanne</td>
<td>82.2B</td>
<td>28.8±0.6B</td>
<td>31.9±0.8CD</td>
<td>9.9±0.3</td>
<td>9.7±0.2</td>
</tr>
<tr>
<td>Sémillon</td>
<td>80.2B</td>
<td>27.6±0.4B</td>
<td>30.2±0.5BC</td>
<td>10.1±0.3</td>
<td>9.2±0.2</td>
</tr>
<tr>
<td>Statistic</td>
<td>13.07a</td>
<td>33.10b</td>
<td>27.6b</td>
<td>9.6b</td>
<td>6.6b</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.023</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Values (mean±s.e) in each column with the same letter are not significantly different (*p*>0.05)

*a* = Pearson $\chi^2$

*b* = Kruskal-Wallis test
Table 2.5 Female sex ratio, females mating success, delay in egg laying, and adult duration of *E. postvittana* reared on different varieties of *V. vinifera*

<table>
<thead>
<tr>
<th>Larval Food</th>
<th>Female sex ratio (%)</th>
<th>Females mating success (%)</th>
<th>Delay in egg laying (days)</th>
<th>Adult duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control</td>
<td>55.6</td>
<td>80</td>
<td>2.1±0.1</td>
<td>11.2±0.6</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>48.5</td>
<td>92</td>
<td>1.9±0.1</td>
<td>14.0±0.6</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>52.5</td>
<td>81</td>
<td>2.3±0.2</td>
<td>12.9±0.6AB</td>
</tr>
<tr>
<td>Merlot</td>
<td>40</td>
<td>87</td>
<td>1.9±0.2</td>
<td>12.9±0.5AB</td>
</tr>
<tr>
<td>Marsanne</td>
<td>52.7</td>
<td>83</td>
<td>2.1±0.1</td>
<td>11.5±0.8A</td>
</tr>
<tr>
<td>Sémillon</td>
<td>40.5</td>
<td>86</td>
<td>2±0.1</td>
<td>12.8±0.4AB</td>
</tr>
<tr>
<td>Statistic</td>
<td>5.24a</td>
<td>7.68b</td>
<td>2.04a</td>
<td>13.1b</td>
</tr>
<tr>
<td>p</td>
<td>0.39</td>
<td>0.17</td>
<td>0.72</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values (mean±s.e) in each column with the same letter are not significantly different (*p*>0.05)

`a` = Pearson χ²

`b` = Kruskal-Wallis test

Table 2.6 Number of adults emerged, number of female participated which contributed to the next generation, female fitness index of *E. postvittana* reared on different varieties of *V. vinifera*.

<table>
<thead>
<tr>
<th>Larval Food</th>
<th>No. of adults emerged (n) (mean ± s.e)</th>
<th>No. of female produced (n) (mean ± s.e)</th>
<th>Female fitness Index (mean ± s.e)</th>
<th>Kruskall-Wallis tests on fitness index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>157.2±21</td>
<td>87.4±11.2</td>
<td>69.9±9.0</td>
<td>A</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>363.2±20.6</td>
<td>176.1±10.0</td>
<td>158.4±9.0</td>
<td>B</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>312.9±33.5</td>
<td>164.3±17.6</td>
<td>133.1±14.2</td>
<td>BC</td>
</tr>
<tr>
<td>Merlot</td>
<td>294.9±30.5</td>
<td>117.9±12.2</td>
<td>102.4±10.6</td>
<td>DC</td>
</tr>
<tr>
<td>Marsanne</td>
<td>198.3±33.6</td>
<td>103.1±16.9</td>
<td>85.6±14.0</td>
<td>AD</td>
</tr>
<tr>
<td>Sémillon</td>
<td>260.4±27.8</td>
<td>134.4±14.5</td>
<td>117.3±12.5</td>
<td>C</td>
</tr>
</tbody>
</table>

Values (mean±s.e) in each column with the same letter are not significantly different (*p*>0.05)
Figure 2.1 Mean number of eggs laid by *E. postvittana* reared on different varieties of *V. vinifera*. Bars with same capital letter are not significantly different (*p* > 0.05)

Figure 2.2 Mean larval emergence hatching from eggs laid by *E. postvittana* reared on different varieties of *V. vinifera*. Bars with same capital letter are not significantly different (*p* > 0.05)
Figure 2.3. Visual representation of the results of the stepwise discriminant analysis: position of five cultivars (○, Control; ▲, Chardonnay; ▼, Marsanne; □, Merlot; △, Sauvignon Blanc; □, Semillon) on the plane defined by the canonical variables 1 and 2. The size of the circle corresponds to a 95% confidence limit for the mean.
This page is intentionally left blank
4.1. Foliar chemistry differs among varieties of *V. vinifera*

The host-plant quality as indicated by nitrogen and carbohydrate contents, and presence of feeding inhibitors such as phenolics form the basis for feeding specialization in an insect (Bernays & Chapman, 1994). Moreover, variation in host-plant quality either positively or negatively affects the performance of plant-feeding Lepidoptera (Ricklefs, 2008). In the present study, the influence of varietal difference on the life-history performance of *E. postvittana* appears to be linked to leaf-nitrogen content. Life-history performance of *E. postvittana* increased when the larvae were fed on the leaves of Chardonnay, which had greater leaf-nitrogen content. Other factors, such as nutrients, total carbohydrates, and phenols in the leaves of Chardonnay, Sauvignon Blanc, Merlot, Sémillon, and Marsanne did not appear to influence *E. postvittana* performance.

Nitrogen is a key limiting nutritional element for leaf-feeding Lepidoptera (Awmack & Leather, 2002). For instance, metabolites necessary for egg yolk, such as essential amino acids vital for egg-protein build-up, in some plant-feeding insects depend exclusively on the nitrogen content in their larval diet (Stockhoff, 1993; O’Brien *et al.*, 2002; Chen *et al.*, 2008). Dietary nitrogen content is known to influence aphid performance (Abisgold *et al.*, 1994). In the present study, the leaves of Chardonnay have been found to include high levels of nitrogen contents, and therefore Chardonnay could be considered the most appropriate host for the best performance of *E. postvittana*. Leaves of Marsanne have the lowest nitrogen levels compared with the varieties tested in this study, resulting in poor larval development and adult performance of *E. postvittana*. Augmentation of soil nutrients often improve the nitrogen concentration of the plant, resulting in higher consumption rates and better life-history performance in generalist Lepidoptera (Lindroth & Kinney 1998; Prudic *et al.*, 2005). Host plants grown with inorganic N attract more individuals of *Bemisia tabaci* (Hemiptera: Aleyrodidae) for oviposition (Bentz *et al.*, 1995), stimulate fecundity, and improve life-history performance (Arancon *et al.*, 2007).
4.2. Varietal differences influence life-history performance of *E. postvittana*

Diet breadth in plant-feeding insects regulates adult fecundity and offspring performance. Plant-feeding insects use plants to maximize their fitness and also that of the offspring (Jaenike, 1990). The balance between phagostimulation and deterrent compounds regulates host range in polyphagous insects (Bernays & Chapman, 1994). Nutritional quality of host-plant(s) used by the larvae and adults is important to regulate life-history performance (Leather, 1995). Longer development time, lower-pupal mass, higher-mortality rate, and greater numbers of instars than usual indicate the suboptimal quality of the host (Frago & Bauce, 2014). For instance, when the larvae of *L. botrana* are reared on different varieties of *V. vinifera* (Moreau et al., 2006) and on other plants (Thiéry & Moreau, 2005) their larval and adult performance varied significantly. Similar results have been shown in *Helicoverpa armigera* (Lepidoptera: Noctuidae), when reared on the leaves of different varieties of *Glycine max* (Fabales: Fabaceae), with varying larval-development periods, survival and fecundity rates, and adult life span (Naseri et al., 2009).

Larvae of the Lepidoptera are voracious feeders and can utilize plant materials of varying nutritive values. *Epiphyas postvittana* feeds on more than 500 species belonging to 121 plant families (Suckling & Brockerhoff, 2010) including several varieties of *V. vinifera* (Paull, 2008). Although Chardonnay is the most-susceptible variety, no variety is known to be 100% resistant (Paull, 2008; Weeks & Pitman, 2012). My results of female-fitness index of *E. postvittana* show that the larvae reared on amended diet+Chardonnay showed the greatest female fitness indicating it to be the most preferred host. In this study, I demonstrated that *V. vinifera* varieties influence the development time of larvae, and consequently alter the pupal mass. Larvae reared on amended diet+Chardonnay developed quickly into heavier pupae, matching with the findings of Danthanarayana et al., (1995). In contrast, extended larval development time when reared on Marsanne resulted in low pupal mass and fecundity rate. Pupal mass is one critical component used to approximate the performance of an insect (Liu et al., 2004). The female pupae produced by larvae fed on amended diet+Chardonnay were significantly heavier than that of pupae produced by larvae reared on amended diet+Marsanne or +Merlot. This
finding supports that Chardonnay is the most suitable variety for *E. postvittana* larvae than the other varieties tested in this study.

My results confirm that the male and female reproductive outputs are significantly influenced by larval-diet quality. The present study also substantiates the outcomes of an earlier study of Paull (2008) that grape variety affects the survival and population dynamics of *E. postvittana* in Australian vineyards. Since adult females were fed only on water in the present study, the essential amino acids, for example, vital for building the egg yolk, were solely derived from the larval diet (O’Brien *et al.*, 2002). The females reared on amended diet+Marsanne laid significantly fewer number of eggs than those reared on amended diets including leaf materials of other varieties except Sémillon. Feeding on amended diet+Marsanne also resulted in reduced viability of eggs, suggesting the suboptimal quality of this variety as a host, whereas *E. postvittana* adult females reared on amended diet+Chardonnay produced greater number of viable eggs. In this study, development time of male larvae was significantly shorter than that of the female larvae reared on amended and control diets. This protandrous behaviour (emergence of males before females) provides maximum opportunities for copulation and minimizes pre-reproductive period of females (Thiéry & Moreau, 2005). Further the protandrous behaviour could be an evolutionary advantage for the females of *E. postvittana*, which generally have a restricted time window for mating and oviposition, since any delay in mating can result in drop in fecundity and fertility (Foster & Ayers, 1996).

The caveat here is that I used phenological stages to determine the development phase of different varieties of *V. vinifera*. Vine vigour may play a major role in insect performance. However, the impact of plant vigour and nutrition on plant-feeding is still being debated (Koricheva *et al.*, 1998). *Tetranychus pacificus* (Acari: Tetranychidae) infestations tend to be more severe on low vigour *V. vinifera* (Hanna *et al.*, 1997). On the other hand, more vigorous *V. arizonica* was more susceptible to attack by *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae) (Kimberling *et al.*, 1990). Low vigour *V. vinifera* shows low leaf nitrogen, which may play a role in attracting *T. pacificus* (Hanna *et al.*, 1997).
Although *E. postvittana* reared on the amended diet+Marsanne showed long larval-development time, low-pupal mass, less fecundity, and fertility, the present results do not support the belief prevalent among grape growers that Marsanne is a non-preferred host for *E. postvittana*. Survival rate, male-pupal mass, adult life span, sex ratio, and hatching proportion of *E. postvittana* reared on amended diet+Marsanne did not show any statistical difference to amended diets with the other varieties. Leaf quality does not explain why Marsanne supports either no or small populations of *E. postvittana*. Nitrogen level can play a role in larval performance but the other nutrient materials such as amino acids and anti-nutrition such as alkaloids, chitinase, β−1, 3−glucanase need to be factored to arrive at a definitive conclusion.

The role of phytopathogens on the performance of associated performance cannot be neglected (Mondy & Corio-Costet, 2000). The influence of *B. cinerea* on the performance of *E. postvittana* is already been reported (Rizvi & Raman, 2015a, b). Many studies show a mutualistic relationship between Tortricidae and *Botrytis cinerea* (Helotiales: Sclerotiniaceae) (Mondy & Corio-Costet, 2000; Rizvi et al., 2015). Further studies are required to evaluate the role of microbial infection on the population dynamics of *E. postvittana* in the context of different varieties of *V. vinifera*.

5. **CONCLUSION**

In Australian vineyards, some varieties of *V. vinifera* suffer more damage due to infestation by *E. postvittana*. In this study, I have showed that different varieties of *V. vinifera* differently affect larval performance and female fitness of *E. postvittana*. Larvae of *E. postvittana* fed on amended diet+Chardonnay experienced better life-history performance. Amended diet+Marsanne, on the other hand, was the poorest particularly when measured for larval and adult performance. The poor response of *E. postvittana* on Marsanne could be useful in IPM via push—pull strategy by planting Marsanne either near or among the susceptible varieties of *V. vinifera*. The results reinforce the view that variation in reproductive performance of *E. postvittana*
due to larval diet should be considered as one key factor affecting the population dynamics of *E. postvittana*.

6. REFERENCES


Chapter 3

Influence of *Botrytis cinerea* infected leaves of *Vitis vinifera* on the preference of *Epiphyas postvittana* *

*Published paper (details below) presented with minor modifications.
1. INTRODUCTION

Chemical cues mediate plant—insect interactions (Schoonhoven et al., 2005; Bruce et al., 2010). Plant-feeding insects use sensory cues such as olfactory, contact chemoreceptory, and visual to identify and assess the food, mates, predators, competitors, and oviposition sites (Schoonhoven et al., 2005; Tasin et al., 2011). Unlike visual and contact chemoreception, olfactory cues are considered helpful to plant-feeding insects in either recognizing or rejecting the host plants from a distance (Bruce et al., 2005; Hallem et al., 2006). Nevertheless, the role of olfactory cues from the host plant is not yet fully explained (Städler, 2002). That the olfactory cues emitted by a plant can guide a gravid lepidopteran on the suitability of a site for oviposition and nutrition for her progeny remains unresolved. It is already know that olfactory cues are critical for locating an appropriate host for oviposition; once the lepidopteran is on the target plant, the contact cues, coupled with the olfactory cues, influence the lepidopteran in assessing the plant, in enabling it to either progress with the oviposition or not (Foster et al., 1997; Tasin et al., 2011). Based on the sensory cues of a plant-feeding insect, judgment on the appropriateness of a host plant is essential for the success of performance of its progeny (Gripenberg et al., 2010). The gravid female lepidopteran uses the olfactory and visual cues to locate and identify the host plant, but the decision to either accept or reject the plant depends on contact cues, which are often plant-surface compounds and texture (Foster et al., 1997; Mehar et al., 2006).

In the natural environment, several organisms attack plants; among these, insects and fungi are associated with a majority of plants, establishing a three-way interacting system (Chapter 1). In such contexts, the fungi influence the interaction between the insect and the plant, which can be either mutualistic or antagonistic, and, occasionally, neutral as well (Hatcher, 1995; van Dam, 2009). Fungi induce variations in the chemistry of the plant which can modify the volatile profile of the plant (Cardoza et al., 2003b; Raman et al., 2012). Such induced variations in the host-plant volatiles could be recognized by the olfactory receptors of Lepidoptera as either an attractant (Cardoza et al., 2003b) or a deterrent (Tasin et al., 2012).
Botrytis cinerea is a necrotrophic plant pathogen that occurs worldwide inducing the grey-mould disease on Vitis vinifera affecting its leaves and berries (Fournier et al., 2013). Botrytis cinerea commonly occurs in the Australian viticultural regions in varying intensities (Nair et al., 1995). Fungal hyphae actively penetrate the cell and enzymatically digest the leaf cells and alter leaf integrity and physiology (Volpin & Elad 1991; Carlile et al., 2001; Jansen et al., 2009). Infection of V. vinifera by B. cinerea induces the biosynthesis of behaviour-modifying volatiles, which influence the host choice made by different insects infesting V. vinifera (Tasin et al., 2012). Often B. cinerea populations co-occur with the larvae of E. postvittana (Chapter 1); the conidia and hyphae of B. cinerea have been found in the gut of mature larvae of E. postvittana. The larvae also facilitate the dispersal of B. cinerea by transmitting the spores that adhere to it and also by passing via excreta (Bailey et al., 1997; Lo & Murrell, 2000; Chapter 1). The feeding damage inflicted by the larvae of E. postvittana render the berries amenable to B. cinerea infection by creating a settling site for and germination of the conidia (Lo & Murrell, 2000). Whether the larvae derive any benefit from the fungus or whether it is co-occurrence remains unanswered.

Epiphyas postvittana, an extremely polyphagous tortricid, is known to feed on more than 500 plant species belonging to 121 families (Suckling & Brockerhoff, 2010). In the field, female moths lay 300–400 eggs in discrete batches of 4–77 (Danthanarayana, 1975). A female of E. postvittana can lay up to 1500 eggs, but the egg-laying capacity is strongly influenced by the nature of food consumed and temperature experienced during its larval stages (Danthanarayana, 1983).

In the present study, I tested the three-way relationship using the three-way interacting system: Epiphyas postvittana—Vitis vinifera—Botrytis cinerea. Experiments were conducted to address the following questions: (1) whether the females of E. postvittana prefer to oviposit on V. vinifera leaves infected by B. cinerea; and (2) whether the larvae of E. postvittana prefer to feed on V. vinifera leaves infected by B. cinerea. Tactile stimuli indeed play a role in host selection, whereas literature (Reddy & Raman, 2011; Machial et al., 2012) reinforces that olfactory stimuli play the principal role in host-plant selection. Using this dictum, in this study I have considered the olfactory stimuli as the principal and earliest trigger
factor. In securing the answers, I compared the ovipositional preference of *E. postvittana* to *B. cinerea*-infected and uninfected (control) leaves of *V. vinifera* at three levels of infection referred to as ‘mild’, ‘moderate’, and ‘intense’. Adult *E. postvittana* female could therefore use olfactory, tactile, and visual cues to assess the suitability of hosts for oviposition. The effect of odour of *B. cinerea*-infected leaves on ovipositional behaviour of *E. postvittana* was tested by allowing the females to use only olfactory cues. The olfactory response of male *E. postvittana* was also compared with *B. cinerea*-infected and uninfected (control) leaves of *V. vinifera*. The olfactory response of the larvae towards the uninfected (control) leaves and *B. cinerea*-infected leaves of *V. vinifera* was compared. The ability of larvae of *E. postvittana* to feed on *B. cinerea*-infected leaves was also examined.

2. MATERIAL AND METHODS

2.1. Insect culture

Eggs of *E. postvittana* were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O). The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm³) following the method described in Chapter 1. The cultures were maintained at 21±1°C, 60–80% RH, and 16L: 8D regimen. All experiments were performed under identical environmental conditions.

Adult males or females were not exposed to either uninfected (control) or *B. cinerea*-infected *V. vinifera*, prior to the experiments. For all bioassays in this study, only neonate larvae (<4 h after emergence) were used.

2.2. Fungus culture and preparation of spore suspension

Mycelia of *B. cinerea* were isolated from the infected berries of *V. vinifera cv. Chardonnay* collected from the Charles Sturt University vineyard ([CSU], Orange, NSW) in February 2013. Source material was provisionally identified based on microscopic observation of the mycelia and conidia (Khazaeli *et al.*, 2010). *Botrytis cinerea* was later confirmed by Michael Priest (Principal Mycologist & Plant
Pathologist, Department of Primary Industries, Orange, NSW). The determined fungal culture was maintained on potato-dextrose agar ([PDA] Fisher Scientific, Inc., Scoresby, Victoria, Australia) at 22°C and 12L: 12D.

To prepare the spore suspension, fungal cultures with c. 2 week old conidia were flooded with 5 ml of sterile water added with 0.01% Tween 80 (Acros Organics, Geel, Belgium) solution and the surface of the B. cinerea colonies was smeared with a bent platinum wire streaker. The density of the conidial suspension was determined with a Neubauer chamber and adjusted to $10^6$ conidia/ml (Trigiano et al., 2008). This suspension was used for the inoculation of leaves of V. vinifera.

2.3. Plant culture

Forty-nine rootlings of V. vinifera cv. Chardonnay (hereafter, referred to as V. vinifera) obtained from a registered vine nursery in Trentham Cliffs, south-western NSW, were raised in nursery-grade potting mixture (Osmocote, Plus Organics Vegetable & Herb Mix, Sydney, Australia) in plastic pots (20.91 dm$^3$) in an insect-proof glasshouse at CSU at 25–27°C and 16L: 8D in October 2012. All plants were watered uniformly every day. Neither insecticides nor fungicides were applied. At the start of the experiments in April 2013, the raised plants were six months old with several fully expanded leaves. Mature, dark-green leaves (9–12 cm long) were used in all experiments. While considering the use of excised leaves in this experiment, I relied on Schmelz et al., (2001), who indicate that the process of senescence could be delayed in laboratory-based assays, though only modestly, when appropriate care is exercised. Nevertheless, excised leaves have been followed in insect—plant interaction assays even in recent times, e.g., Sharma et al., (2005), and Michel et al., (2010) for reasons of experimental convenience. Peros et al., (2006) had used excised leaves to characterize fungus—plant (Erysiphe necator—V. vinifera) interactions. Keeping the above in view, I used excised leaves, coupled with a damp-filter paper to maintain a reasonable level of moisture in my experiments, thus delaying senescence during experiments.
2.4. Level of infection of *V. vinifera* leaves with *Botrytis cinerea*

Excised leaves (petioles+laminae) of *V. vinifera* were surface sterilized with 1% NaClO solution for 5 min and washed with sterile water (3x). After surface sterilization, leaves were immediately sprayed with the spore suspension on the sterile bench of a horizontal laminar airflow cabinet (HWS120, Clyde–Apac, Sydney, Australia). Control leaves were sprayed with sterile water. Inoculated and control leaves were placed in a zip-lock plastic bag (35x40 cm) supplied with a damp filter paper and incubated at 22°C and 12L: 12D. After 4−6, 9−11, and 14−16 d, the lesions on the *B. cinerea*-infected leaves were evaluated as 5−10% being ‘mild’, 30−60% being ‘moderate’ and 90−100% being ‘intense’, respectively. The infection level of *B. cinerea*-induced lesions was measured in percentage, from the photographs made with a digital camera (D–60, Canon, Tokyo, Japan) and calculating the area of infection using ‘ImageJ’, an open-source image processing software (Abramoff et al., 2004).

2.5. Bioassay of oviposition behaviour

Experiments were conducted using excised *V. vinifera* leaves bearing infection levels as mild, moderate, and intense along with appropriate control leaves. The experiments were conducted in custom-made Pyrex® glass devices (details in the following sections 2.5.1) at 20–22°C, 60−70% RH, and 16L: 8D (four units of 28W slim-line diffuse flush fluorescent light tubes, with an additional plastic sheet acting as a diffuser attached). On completion of every experiment, the custom-made glass devices were washed thoroughly with a commercial-detergent solution, followed by a 70% ethanol rinse, and oven dried at 110°C for eight hours before setting up the next experiment. A two-choice experiment was set up to evaluate the ovipositional preference pattern of *E. postvittana* on *B. cinerea*-infected and uninfected (control) leaves. A no-choice experiment was set up to evaluate the rate of oviposition on *B. cinerea*-infected and uninfected (control) leaves, using an identical but a different glass device. Details of glass devices used in the two experiments are explained in the following paragraphs.
2.5.1. Two-choice experiment

The two-choice experiment was conducted using a custom-made glass device, which consisted of two 1000 ml flasks connected by a horizontal glass tube (30 cm long, 3 cm wide). The diameter of the horizontal tube was chosen in such a way that its ends fitted snugly into the mouths of the flasks; the junctions were sealed tightly with Parafilm®. At the mid-point of the horizontal glass tube, a 2-cm wide circular port was cut for the introduction of insects. Three *B. cinerea*-infected leaves (5–10%) of *V. vinifera* (7.00±0.23g; mean±s.e) were placed in one flask and three control leaves of *V. vinifera* (7.00 ±0.18g; mean ±s.e) were placed in the other. Five gravid *E. postvittana* adults were introduced through the port, allowing the insects to make their choice of either *B. cinerea*-infected or control leaves (Figure 3.1). The port was covered with Parafilm®. After 72 h, the insects, all of which remained alive and active, were removed by disassembling the device. Choices made by the insects were recorded by counting the numbers in either of the flasks and the number of eggs laid on *B. cinerea*-infected and control leaves were counted using a stereo-binocular microscope (S–20, AIS Instrument Services, Croydon, Victoria, Australia). The same procedure was repeated for leaves with 30–60% and 90–100% infection levels, eight times each. In each experiment, the location of infected and control leaves was randomised. Each treatment was replicated 8 times by using four glass devices on each day.

2.5.2. No-choice experiment

This experiment was done following Yan et al., (1999) with little modification. Either two *B. cinerea*-infected leaves (5–10% or 30–60% or 90–100%) or two appropriate control leaves of *V. vinifera* (4.2±0.21g; mean±s.e) were placed inside a 500 ml Pyrex glass conical flask. A gravid *E. postvittana* was introduced into the conical flask and the opening was sealed immediately with Parafilm (Figure 3.2). After 72 h the moths were removed and the eggs laid on the leaves were counted. Each treatment was replicated 10 times.
### 2.5.3. Effect of volatiles on oviposition

In this experiment, ovipositional rate of *E. postvittana* was tested in response to the volatile compounds emitted from *B. cinerea*-infected leaves or appropriate control leaves of *V. vinifera*. A 200 ml Dixie cup with vertical ridges and furrows was used as an ovipositional device. The bottom of each cup was removed, wrapped in plastic wrap, and pierced 30 times using a safety pin to allow the volatiles to pass inside the cup. This cup was then fixed over a 250 ml beaker, which included a damp filter-paper. Either two control leaves or two *B. cinerea*-infected leaves (5–10% or 30–60% or 90–100%) of *V. vinifera* (3.96±0.12 g; mean±s.e) were placed inside the beaker. The junction point of the beaker with the Dixie cup was sealed with Parafilm (Figure 3.3). A 24 h old mated *E. postvittana* female was introduced into the Dixie cup and the cup was covered with Parafilm. After 72 h, moths were removed and eggs deposited on the cup counted. Each treatment was replicated 10 times.

### 2.6. Y—tube experiment

The behavioural responses of adult males *E. postvittana* (24–48 h old) towards *B. cinerea*-infected leaves (5–10% or 30–60% or 90–100%) and appropriate control leaves of *V. vinifera* were measured using a Y-tube olfactometer (OLFM—YT—2425F; Analytical Research Systems, Gainsville, Florida, USA) having a stem (2.5 cm diameter; 15 cm long), which forked into two arms (2.5 cm diameter, 7.5 cm long) (see Figure 7.3, Chapter 7). Three *B. cinerea*-infected (5–10% or 30–60% or 90–100%) (6.89±0.325 g; mean±s.e) and three (7.01±0.152 g; mean±s.e) appropriate control leaves of *V. vinifera* were enclosed in the glass chambers which were connected to the ends of the Y-tube by flexible Teflon tubes. Compressed air with a flow rate of 300 ml/min was allowed to enter the chamber through the Teflon tube inlet and to come out through the outlet. Moths were introduced individually through the downwind end of the Y-tube and observed for 15 min. Moths that remained at the downwind end for 30 min were deemed unresponsive and excluded from the analyses. Female moths were also tested but they did not respond in the Y-tube, so were excluded from the analysis.
2.7. Larval bioassays

2.7.1. Two-choice experiment

This bioassay was conducted to test the preference of neonate larvae of *E. postvittana* towards *B. cinerea*-infected (5−10% or 30−60% or 90−100%) and appropriate control leaves by following Foster and Howard (1999) with some modification. In all experiments, newly-hatched larvae were used (<2h old). In this experiment, a glass tube (30 x 3cm internal diameter) with an opening of 2 cm diameter in the centre was used. One control leaf and one *B. cinerea*-infected leaf were inserted individually at each end of the tube, separated by 6 cm from the midpoint.

Ten newly hatched larvae were gently placed in the tube through the opening, which was immediately sealed with Parafilm (Figure 3.4). After 24 h the position of the larvae was noted. This experiment was repeated 10 times. The same incubation conditions used in the two-choice experiment assaying the oviposition behaviour were used.

2.7.2. Larval acceptance and transmission of conidia of Botrytis cinerea

This bioassay was conducted to test the acceptance and transmission of *B. cinerea* conidia following Foster and Howard (1999) and Bailey *et al.* (1997). An intensely infected (90−100%) *B. cinerea*-infected leaf with sporulating *B. cinerea* or an uninfected (control) leaf was placed in a 9-cm wide Petri plate that included a damp filter paper. A neonate larva raised from the egg stock from the refrigerated egg rafts was placed on each leaf; the edges of each Petri-plate set were sealed with Parafilm (Figure 3.5). After 72 h, either acceptance or mortality of the larva was noted. To determine the presence of conidia of *B. cinerea* on the cuticle of larvae, each larva from control or *B. cinerea*-infected leaf was cleaned in 2 ml sterilized distilled water in a vortex mixer (VM1, Ratek, Victoria, Australia) (Bailey *et al.*, 1997) and the washed water was examined in a light microscope (YS2−H, Nikon, Tokyo, Japan). For internal examination, each larva was surface sterilized with 10% NaClO solution for 1 minutes and then washed with sterilized water (x3) and dissected in a stereo-
binocular microscope (S−20, AIS Instrument Services, Croydon, Victoria, Australia). Gut material of each larva was spread over PDA plate and incubated at 22°C in dark for 72 h to observe whether *B. cinerea* colonies were established. The experiment was repeated 30 times.

2.8. **Statistical analysis**

In the two-choice experiment the numbers of eggs laid on *B. cinerea*-infected and appropriate control leaves were subjected to a paired sample ‘t’ test. Number of eggs from the no-choice experiment and effect of volatile experiment were analyzed using an independent sample ‘t’ test. To get different levels of infection, differently aged leaves were required, so a separate control was used for each infection level and therefore individual ‘t’ tests were considered appropriate. Data from the Y-tube olfactometer and choice of the female moths for *B. cinerea*-infected or control leaves were analysed using Chi-square test. Analyses were conducted with SPSS v17.0 (1993–2007). Graphs were generated in MS Excel 2013.

3. **RESULTS**

3.1. **Bioassay of oviposition behaviour**

3.1.1. *Two-choice experiment*

In the two-choice experiment, when visual, olfactory, and tactile sensory cues were available for *E. postvittana* adults, the females showed a significant preference for control leaves (70%) compared with the intensely infect (30%, N=40, \(\chi^2=6.4, p=0.01\), Figure 3.6). In contrast, no significant differences in the preference for control leaves (45%) compared with mildly infected leaves (55%, N=40, \(\chi^2=0.4, p=0.53\), Figure 3.6) and for control leaves (63%) compared with the moderately infected leaves (37%, N=40, \(\chi^2=2.5, p=0.11\), Figure 3.6) occurred.

For oviposition, mild infection of leaves did not significantly deter the oviposition of *E. postvittana* (control vs infected, 437 vs 375, t=0.83, *p*=0.47, Figure 3.7), but the moderately and intensely infected leaves
Figure 3.1 Custom-made glass device (horizontal tube connected to two 1000 ml flasks) for the two-choice experiment of adults of *Epiphyas postvittana* (not to scale) (f–flask, hgt–horizontal-glass tube, il–infected leaves, m–moth, pe–port of entry, ps–parafilm seal, ul–uninfected leaves).

Figure 3.2 Custom-made glass device for the no-choice experiment of adults of *Epiphyas postvittana* (not to scale) (f–flask, il–infected leaves, m–moth, ps–parafilm seal, ul–uninfected leaves).

Figure 3.3 Custom-made device to test the effect of volatiles on oviposition behaviour of *Epiphyas postvittana* (not to scale) (b–beaker, d–Dixie cup, il–infected leaves, m–moth, ps–parafilm seal, pw–plastic wrap, ul–uninfected leaves).
Figure 3.4 Custom-made glass device for the two-choice experiment of larvae of *Epiphyas postvittana* (not to scale) (hgt—horizontal-glass tube, il—infected leaf, pe—port of entry, ps—parafilm seal, ul—uninfected leaf).

Figure 3.5 Petri-plate pair test for the larval acceptance and transmission of conidia of *Botrytis cinerea* (not to scale) (dfp—damp filter paper, pp—Petri plate, ul—uninfected leaf, il—infected leaf).
significantly deterred oviposition (moderate infection, control vs infected, 451 vs 185, t=2.78, p=0.03, Figure 3.7; intense infection, control vs infected, 599.3 vs 108, t=9.89, p = 0.001, Figure 3.7).

3.1.2. No-choice experiment

Gravid females oviposited on either B. cinerea-infected leaves (5−10% or 30−60% or 90−100%) or appropriate control leaves. No egg occurred at the B. cinerea-infected sites on V. vinifera leaves. No significant difference occurred in the number of eggs laid on control leaves compared with the mildly infected leaves (control vs infected, 264.2 vs 214, t=2.03, p=0.08, Figure 3.8). Significantly fewer eggs were laid on moderately and intensely infected leaves compared with the control leaves (mild infection, control vs infected, 250 vs 85, t=4.19, p=0.01, Figure 3.8; intensely infected, control vs infected, 208 vs 36, t=5.841, p=0.001, Figure 3.8).

3.2. Effect of volatiles on oviposition

In a no-choice bioassay context, gravid females oviposited on the smooth surfaced, corrugated wall of the Dixie cup, in the response to the volatile compounds emitted from B. cinerea-infected leaves (5−10% or 30−60% or 90−100%) or appropriate control leaves. Volatiles from mild and moderate infected leaves did not significantly deter the oviposition of E. postvittana (mild infection, control vs infected, 396 vs 403, t=0.56, p=0.58, Figure 3.9; Moderate infection, control vs infected, 356 vs 339, t=0.65, p=0.53, Figure 3.9). Significantly fewer eggs were laid on the wall of cups that contained volatiles from intensely infected leaves compared with the control leaves (control vs infected, 357 vs 285, t=2.27 p=0.04, Figure 3.9).

3.3. Y-tube experiment

Male moths exhibited a significant-level of preference for the odour of control leaves (67.5%) compared with intensely infected leaves (32.5%, N=40, $\chi^2=4.9$, p=0.03, Figure 3.10). On the contrary, no significant difference occurred in the preference for control leaves (56.6%), compared with mildly infected leaves (43.4%, N=40,
\( \chi^2 = 0.53, p = 0.45 \), Figure 3.10). No significant difference occurred in the preference for control leaves (63.6%) compared with moderately infected leaves (36.6%, \( \chi^2 = 2.13, p = 0.14 \), Figure 3.10). Female moths (\( n = 10 \)) did not respond to any treatment in this experiment.

3.4. Larval bioassays

3.4.1. Two-choice experiment

The neonate larvae of \( E. postvittana \) (\( n = 100 \)) demonstrated a significant preference for the mildly infected leaf (61%) compared with control leaf (39%, \( \chi^2 = 5.33, p = 0.02 \), Figure 3.11) and for moderately infected leaf (60%) compared with control leaf (40%, \( \chi^2 = 3.84, p = 0.049 \), Figure 3.11). Whereas the larvae showed no significant difference in their preference when allowed to choose either the intensely infected leaf (49%) or the control leaf (51%, \( \chi^2 = 0.05, p = 0.82 \), Figure 3.11).

3.4.2. Larval acceptance and transmission of conidia of \( B. cinerea \)

The introduced neonate larvae of \( E. postvittana \) on control leaf or \( B. cinerea \)-infected leaf remained alive and active for the next 72 h. External and internal body examination of the larvae fed on control leaf demonstrated that they did not carry any conidia or hyphae of \( B. cinerea \). Whereas, the larvae fed on the \( B. cinerea \)-infected leaf carried the conidia of \( B. cinerea \) on their body surface (Table 3.1). Internal examination demonstrated that the larvae fed on hyphae and conidia of the fungus, since these were found in the gut of the larvae. Conidia from the gut material of all larvae, fed on infected leaves, tested positive and resulted in the growth of \( B. cinerea \) on the PDA plate.

4. DISCUSSION

The test of preference patterns of \( E. postvittana \) indicates that the moderately and intensely \( B. cinerea \) infected leaves of \( V. vinifera \) significantly deter oviposition,
Figure 3.6 Response of females of *E. postvittana* in a ‘two-choice’ experiment towards uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C]. Levels of infection expressed in percentage. *indicates a significant difference (*p*<0.05, NS—Not significant).

Figure 3.7 Mean number of eggs laid by *E. postvittana* in a ‘two-choice’ experiment with uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C]. Levels of infection expressed in percentage. *indicates a significant difference (*p*<0.05, **p**<0.001, NS—Not significant).
Figure 3.8 Mean number of eggs laid by *E. postvittana* in the ‘no-choice’ experiment with uninfected (control) leaves (■) or *B. cinerea*-infected leaves (□) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C]. Levels of infection expressed in percentage. *indicates a significant difference (*p < 0.05, ***p < 0.001, NS–Not significant).

Figure 3.9 Mean number of eggs laid by *E. postvittana* on corrugated wall of the Dixie cup, in response to the volatile compounds emitted from uninfected (control) leaves (■) or *B. cinerea*-infected leaves (□) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C] in ‘effect of volatile on oviposition’ experiment. Error bars denote SEM (n=10). Levels of infection expressed in percentage. *indicates a significant difference (*p < 0.05, NS–Not significant).
Figure 3.10 Response of adult male *E. postvittana* in a ‘Y-tube’ experiment toward uninfected (control) leaves (Ⅲ) and *B. cinerea*-infected leaves (Ⅱ) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C]. Levels of infection expressed in percentage. * indicates a significant difference (*p*<0.05, NS–Not significant).

Figure 3.11 Response of larvae of *E. postvittana* in a ‘two-choice’ experiment toward uninfected (control) leaves (Ⅲ) and *B. cinerea*-infected leaves (Ⅱ) of *V. vinifera*: 5–10% (Mildly infected) [A], 30–60% (Moderately infected) [B], 90–100% (Intensely infected) [C]. Levels of infection expressed in percentage. * indicates a significant difference (*p*<0.05, NS–Not significant).
whereas the larvae exhibit a significant level of preference for mildly (5–10%) and moderately (30–60%) *B. cinerea*-infected leaves, but, on the contrary, show no significant level of preference for the intensely (90–100%) infected leaves. Viable conidia occurred in the gut and on the cuticle of the larvae that had fed on *B. cinerea*-infected leaves, thus indicating that the larvae spread the conidia among the healthy leaves and berries.

Infection by fungi induces changes in the nutritional status of the host plant, which, in turn, can also influence the volatiles emitted by the infected plant (Cardoza *et al*., 2002; Tasin *et al*., 2012). These changes influence the olfactory response of the Lepidoptera by either inhibiting or enhancing attraction and/or oviposition (Cardoza *et al*., 2003b; Dötterl *et al*., 2009; van Dam 2009; Tasin *et al*., 2011). In the present study, the decision made on the host quality for oviposition by the gravid *E. postvittana* was influenced by the stimuli that arose from *V. vinifera* infected by *B. cinerea*. *Vitis vinifera* leaves infected by *B. cinerea* elicit avoidance behaviour in adult female *E. postvittana* and thus prevent oviposition. Although the mild (5–10%) level of infection by *B. cinerea* did not significantly deter oviposition, the moderate (30–60%) and intense (90–100%) levels of infection significantly deterred oviposition. Avoidance of leaves of vascular plants infected by a fungal pathogen for oviposition by a plant-feeding insect is an adaptive strategy for the fitness of the offspring (Niinemets *et al*., 2013). An infected plant could still be a source of food for the Lepidoptera, since the associated fungus could improve insect nutrition either by breaking the complex materials into simpler forms or by decreasing the level of defense compounds (Cardoza *et al*., 2003a; Hatcher, 1995). However, the time difference between egg laying and hatching could increase the level of infection and therefore, the potential larval food could rapidly deteriorate in quality (Tasin *et al*., 2012). In such contexts, the avoidance of moderately and intensely *B. cinerea*-infected sites on the leaves of *V. vinifera* by the ovipositing adults of *E. postvittana* suggests that it is an adaptive strategy of *E. postvittana* for ensuring suitable feeding sites for their offspring. Odours from leaves with an intense level of infection (90–100%) also deterred the male moth, which could positively affect mating behaviour, since the male moths use plant volatiles to distinguish environments where the likelihood to find females is higher (Ansebo *et al*., 2004).
Table 3.1 Percentage acceptance of *B. cinerea*-infected *V. vinifera* leaves by the larvae of *E. postvittana* and its percentage which carried viable conidia of *B. cinerea*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larvae examined</th>
<th>Acceptance (%)</th>
<th>Percentage of larvae carrying conidia of <em>Botrytis cinerea</em></th>
<th>Body surface</th>
<th>Gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected leaf (90–100%)</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>control leaf</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
In the ‘no-choice’ bioassays, no eggs were laid at the *B. cinerea*-infected sites on *V. vinifera* leaves. *Epiphyas postvittana* laid significantly fewer eggs on moderately (30–60%), and intensely (90–100%) *B. cinerea*-infected leaves, which reinforces the findings made in the two-choice experiment reported in this paper. *Botrytis cinerea* is known to degrade hydrocarbons and to release volatile compounds, such as 3-methyl-1–butanol, which is an indicator of decaying plant tissues (Magan & Evans, 2000; Tasin *et al*., 2012). These physiological changes, consequent to tissue decay, and the release of volatiles is sensed by the gravid *E. postvittana*, which is repelled, and in turn deterred from oviposition. Applying the preference–performance hypothesis (Gripenberg *et al*., 2010), I interpret here that the decayed quality of the leaves, which the larvae would be encountering on hatching, would not be optimal for their performance. A similar result was observed for gravid females of *L. botrana* which oviposited on 1–3 day old *B. cinerea*-infected berries of *V. vinifera* but significantly deterred ovipositing on 7–9 day old *B. cinerea*-infected berries of *V. vinifera* (Tasin *et al*., 2012).

Tactile stimuli including surface texture play a secondary role in host selection, especially for oviposition (Rojas *et al*., 2003; Foster & Howard, 1998). Depending on the characteristics of their host plants or the species-dependent optimal conditions for survival of the eggs, females prefer smooth, hairy or rough surfaces for oviposition (Renwick & Chew, 1994). Ovipositional behaviour in *E. postvittana* is strongly influenced by tactile stimuli (Foster *et al*., 1997). The gravid *E. postvittana* prefers to oviposit on a smooth surface with varicose texture, rather than on rough and hairy surfaces. *Epiphyas postvittana* laid more eggs on the adaxial leaf surfaces which bears fewer hairs (Figure 3.12 a, b) than abaxial surface (Tomkins *et al*., 1991). Where gravid *E. postvittana* were allowed to lay eggs on smooth plastic Dixie cup with varicose structure in the presence of fungi at mild, moderate, and intense infection levels, only the intense-level of infection (90–100%) deterred the oviposition of *E. postvittana* significantly ($p<0.05$). The number of eggs laid on the Dixie cup at all infection levels (mild, moderate, and intense) including the control leaves were greater than the number of eggs laid on leaves at the same infection levels in a ‘no-choice’ bioassay (*cf*. Figures 3.8 and 3.9). Based on this observation, I explain that tactile stimuli may influence oviposition in *E. postvittana*. The relative differences between the number of eggs laid on control leaves and *B. cinerea*
infected leaves at all infection levels (mild (50.70), moderate (161.32), and intense (172.02) were higher in the ‘no-choice’ bioassay (Figure 3.8) compared to the ‘effect of volatile’ bioassay (mild (4.89), moderate (19.95), and intense (80.11)) (Figure 3.9). Therefore, I infer from these data that the mycelial bed of *B. cinerea*, which is apparently similar to trichomatous leaf surfaces (Figure 3.12 c, d), may influence the ovipositional behaviour of *E. postvittana*. My results support the findings of other studies, where it has been suggested that the texture and tactile features of the leaf surface are key triggers factors and influence oviposition (Rojas *et al.*, 2003; Foster & Howard, 1998; Tomkins *et al.*, 1991). Chemical and visual cues are likely to play an important role on ovipositional behaviour of *E. postvittana* which needs to be explored.

The larvae of *E. postvittana* showed significant attraction towards mildly and moderately *B. cinerea*-infected leaves of *V. vinifera* in the two-choice experiment; notwithstanding the fact the experiments have been done using excised leaves. However, no significant difference occurred, when the infection level was intense. This suggests that the larvae of *E. postvittana* have mutualistic relationship with *B. cinerea*. Senescence of the infected leaves of *V. vinifera* releases nitrogenous material for *E. postvittana* larvae and this is accelerated due to infection by *B. cinerea*. Similar results have been reported for the larvae of *L. botrana* reared on the fruits of *V. vinifera* and *Pyrus malus* (Rosaceae) infected by *B. cinerea* (Savopoulou-Soultani & Tzanakakis, 1988; Mondy *et al.*, 1998a, b; Mondy & Corio-Costet, 2000). The intensely-infected leaves of *V. vinifera* deteriorate more rapidly, and that possibly explains why such leaves are unattractive to the larvae of *E. postvittana*. Mutualistic relationship between insects and fungi associated with vascular plants is common among diverse insects (Svoboda *et al.*, 1994; Mondy *et al.*, 1998a), where the fungi act as a source of nitrogen, carbohydrates, vitamin B, and sterols (Hatcher, 1995).

Whether *B. cinerea* has a role in the development of *E. postvittana* by acting as a food source or by supplementing nutrients and sterols remains to be verified. In the present study, I used neonate larvae of *E. postvittana* in
Figure 3.12 Surface views of *V. vinifera* leaves (Photo by Syed Rizvi).
A. Adaxial view (Bar = 1 mm), B. Abaxial view (Bar = 1 mm), C. Mycelial bed of *Botrytis cinerea* on adaxial side (Bar = 1 mm), D. Conidia (black heads) of *B. cinerea*) (Bar = 1 mm) (Arrowhead – trichome)
experiments to test whether the *B. cinerea*-infected leaves were preferred by them. All the larvae of *E. postvittana* were alive after 72 hours, feeding on *B. cinerea*-infected leaves indicating that the larvae accepted *B. cinerea* as a part of their diet. Dissection of the first-instar larvae showed viable conidia in their gut, which reinforces that the larvae do not exclude *B. cinerea* during feeding on the leaf tissues of *V. vinifera*. Moreover, the larvae transport viable conidia on their body surface, thus distributing the fungus to new sites on the plant. The results of the present study are similar to those of Bailey *et al.* (1997), who found viable conidia of *B. cinerea* on the cuticle, in the gut, and the faeces of the fourth-instar larvae of *E. postvittana*.

5. CONCLUSION

In this study, I show that the gravid females of *E. postvittana* avoid *B. cinerea*-infected leaves during oviposition and the rate of oviposition is inversely related to the levels of infection. In contrast, the larvae of *E. postvittana* prefer to feed on mild and moderately *B. cinerea*-infected leaves. Incidence of viable conidia of *B. cinerea* in the gut and on the body surface of *E. postvittana* suggest that *E. postvittana* plays a role in spreading the conidia of *B. cinerea* on the leaves of *V. vinifera*. The co-occurrence of the *B. cinerea* and larvae of *E. postvittana* on *V. vinifera* and the preference of the larvae of *E. postvittana* towards *B. cinerea*-infected leaves indicate a possible mutualism, which remains to be verified.

6. REFERENCES


Mondy, N. and Corio-Costet, M.F. (2000) The response of the grape berry moth (Lobesia botrana) to a dietary phytopathogenic fungus (Botrytis cinerea):


Chapter 4

Oviposition behaviour and life-history performance of *Epiphyas postvittana* on the leaves of *Vitis vinifera* infected with *Botrytis cinerea* *

*Published paper (details below) presented with minor modifications.

This page is intentionally left blank
1. **INTRODUCTION**

Infection by pathogenic fungi alters the chemistry of host plants and influences oviposition preference and larval performance in plant-feeding insects (Mondy & Corio-Costet, 2000; Raman et al., 2012; Rizvi et al., 2015a). Fungal infections can interfere with the biosynthetic pathways in the plant in various ways. Some lead to a decrease in the production of insect-attracting volatiles (Dötterl et al., 2009), whereas others produce several behaviour-modifying volatiles (Tasin et al., 2011). Fungal infection of a plant can suppress its direct defence capability against plant-feeding insects by modifying the plant’s secondary metabolism (Thaler et al., 1999) and the nutritional quality of the plant, thus rendering the plant susceptible for insect colonization (Cardoza et al., 2003). On the other hand, fungal infection can deteriorate the nutritional quality of the plant and influence the plant-feeding insect’s development negatively (Tasin et al., 2012).

Olfactory cues are critical for searching a suitable host from a distance for oviposition but once the insect lands on the target plant, the post-landing cues, viz., contact, together with volatiles can influence the insect for an assessment of the plant and can either deter or stimulate oviposition (Renwick & Chew, 1994; Foster et al., 1997; Tasin et al., 2011). Based on the sensory cues of a plant-feeding insect, judgment on the appropriateness of a host plant is essential for the success of progeny performance (Gripenberg et al., 2010). Optimization theory of host searching suggests that the gravid female should choose those plants, which maximize the fitness of her progeny (Jaenike, 1978), although evidences negating Jaenike (1978) also exist (Larsson & Ekbom, 1995; Leyva et al., 2000). The choice made by the juveniles of insects for food can substantially differ from the adults, particularly when the larvae and adults feed on different plant parts (Mayhew, 1997).

In general, the life-history traits of the taxa of Tortricidae (Lepidoptera) vary, when host quality varies. Insect life-history performance traits, such as longer development time, lower pupal mass, higher mortality rate, and greater numbers of instar stages than usual indicate the suboptimal quality of the host (Frago & Bauce, 2014). For instance, *Epiphyas postvittana*, when reared on young leaves of *V. vinifera* and on the leaves and fruits of varieties ‘Valencia’ and ‘Washington Navel’
of *Citrus aurantium* (Rutaceae) *ex-situ*, their survival rates and developmental periods vary significantly (Mo *et al.*., 2006). Similar results have been shown in *Argyrotaenia sphaleropa* (Lepidoptera: Tortricidae) when reared on the leaves of *V. vinifera* var. Muscat of Hamburg and *Malus domestica* var. Red Delicious (Rosales: Rosaceae) with varying developmental periods and survival rates (Bentancourt *et al.*., 2003). High levels of hydrolyzable sugars in plant tissues often have negative effects on the development of plant-feeding insects, because these sugars affect the quality of other nutrients, thus requiring the insects to increase their consumption rate offsetting deficiencies (Bartlet *et al.*, 1990). When individuals of *Choristoneura occidentalis* (Leipodoptera: Tortricidae) were reared on a synthetic diet, their population growth rates were negatively influenced by >10% concentrations of sucrose (Clancy, 1992).

Nevertheless, in three-way interacting systems involving an insect and a plant-pathogenic fungus, both developing on the same plant, the insects generally gain in terms of their growth and metabolism (see Chapter 1). Leaf tissues of *Arachis hypogaea* (Fabales: Fabaceae)-infected with *Sclerotium rolfsii* (Atheliales: Atheliaceae) showed significantly greater levels of soluble sugars but lower starch content and total soluble phenolics compared with uninfected leaves of *A. hypogaea*. *Spodoptera exigua* showed significantly greater survival rates, produced significantly heavier pupae, and had shorter time span between the last larval instar and pupation, when fed on the foliage of *A. hypogaea* infected with *S. rolfsii* (Cardoza *et al.*, 2003). For instance, in the *Botrytis cinerea*–*Lobesia botrana* (Lepidoptera: Tortricidae)—*V. vinifera* interacting system, *L. botrana* have been demonstrated to gain from *B. cinerea* by acquiring sterols, which enhanced their ability to metamorphose rapidly gaining greater biomass (Mondy & Corio-Costet, 2000). In another similar relationship among *Cydia pomonella* (Lepidoptera: Tortricidae)–*Metschnikowia andauensis* (Saccharomycetales: Metschnikowiaceae)–*M. domestica*, the larvae have been shown to remain attracted towards *M. andauensis*-infected *M. domestica* fruits along with low-mortality rate and a better pupation number (Witzgall *et al.*, 2012). The nutrient composition and levels of fungal infection in plants influence the fitness and associated performance parameters, such as growth, development, survival, and reproduction (Lindroth *et al.*, 1991; Rizvi *et al.*, 2015b). These findings indicate that the Lepidoptera gain from fungi associated with plants, as demonstrated by
accelerated growth rate, although in essence the insect can feed solely on plant tissue and still survive and reproduce.

Keeping the above in view, I expect that *E. postvittana* co-occurs with *V. vinifera* infected by *B. cinerea*, and that for a better larval performance and oviposition behaviour of *E. postvittana*, *V. vinifera–B. cinerea* interacting system is imperative. To test this, I sought answers to the following questions: (1) whether the females of *E. postvittana* prefer ovipositing on *V. vinifera* infected by *B. cinerea*, (2) whether *B. cinerea*-infected leaves positively influence larval performance of *E. postvittana*, and (3) whether the larvae of *E. postvittana* can survive and develop feeding exclusively on *B. cinerea*. To secure answers, I tested the oviposition preference of *E. postvittana* using *B. cinerea*-infected and uninfected leaves of *V. vinifera* in a free-choice oviposition experiment. I also explored the oviposition rate of *E. postvittana* in a no-choice experiment endowed with either *B. cinerea*-infected or uninfected leaves of *V. vinifera*. To test the larval performance with or without *B. cinerea*, I raised the larvae of *E. postvittana* on uninfected and *B. cinerea*-infected *V. vinifera* leaves, using standard plant-nutrient solution (e.g., Knop’s solution) simulating near natural conditions as well as on standard synthetic culture media (e.g., potato-dextrose agar [PDA], Murashige and Skoog medium [M–S medium]). To verify the survival and development rate of the larvae of *E. postvittana*, I reared the larvae on *B. cinerea* cultured on PDA and M–S medium, without *V. vinifera*.

2. MATERIAL AND METHODS

2.2. Insect culture

Eggs of *E. postvittana* were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O) The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm³) following the method described in (see Chapter 2). The cultures were maintained at 21±1°C, 60–80% RH, and 16L: 8D regimen. All tests were performed under identical environmental conditions.
Only those females, which laid 5–10 eggs, were used in the oviposition bioassays. Adult males and females were not exposed to either uninfected or *B. cinerea*-infected leaves of *V. vinifera*, prior to experimentation.

2.3. **Preparation of conidial suspension**

The conidial suspension was prepared in sterile water following the method described in Chapter 3) and adjusted to $10^6$ conidia/ml.

2.4. **Inoculation of *V. vinifera* leaves with *B. cinerea***

Sixty plants of *V. vinifera* were raised in plastic pots as mentioned earlier in Chapter 3 Six-week old plants were used in the trials. The conidial suspension was sprayed using a hand-held atomizer to infect the leaves of *V. vinifera*. Uninfected (control) leaves were sprayed with sterile water identically. The inoculated and uninoculated plants were covered with a plastic bag to enhance humidity and placed in an environmental chamber (Thermoline L+M, Thermoline Scientific, NSW, Australia) (23°C, 70% RH, and 16L: 8D). Those plants which manifested disease symptoms were used in the experiments. The disease symptoms on *V. vinifera* leaves were estimated by measuring in percentage from photographs made with a digital camera (D–60, Canon, Tokyo, Japan) and calculating the area of infection using *ImageJ*, a public-domain image processing software (Rizvi et al., 2015a). Those plants which showed 10—30% infection level were used in the oviposition bioassays.

2.5. **Oviposition behaviour**

2.5.1. **Free-choice experiment**

Three uninfected and three *B. cinerea*-infected plants were placed in an aluminium-mesh cage (200x80x80 cm$^3$). The plants were placed 10 cm away from the edge of the cage and were distributed in two rows, separated by 40 cm between plants. Uninfected and infected plants were placed alternately (Figure 4.1). Ten gravid
females of *E. postvittana* were released at the midpoint of the cage allowing the insects to choose either *B. cinerea*-infected or uninfected plants for oviposition. The set-up was left undisturbed for 72 h. After 72 h, the plants were checked for eggs and the number of eggs was counted under a stereo-binocular microscope. This experiment was repeated six times. In each replicate new plants were used and the position of the infected and uninfected plants were randomized.

2.5.2. *Two-choice experiment*

One *B. cinerea*-infected and one uninfected plants were individually placed in two glass containers (60x30x30 cm³) which have tiny holes on one side to facilitate the passage of air. The two containers were connected by a horizontal glass tube (30 cm long, 3 cm diameter) (Figure 4.2). At the mid-point of the horizontal glass tube, a 2-cm wide circular port was cut to introduce the moths (Figure 4.2). Five gravid individuals of *E. postvittana* were introduced through the port, enabling them to choose either *B. cinerea*-infected or uninfected plant for oviposition. Soon after introduction, the port was closed using a Parafilm® strip. After 72 h, the insects were removed. The choices made by the insects were recorded and the numbers of eggs laid on *B. cinerea*-infected and uninfected *V. vinifera* were counted under a stereo-binocular microscope. This experiment was repeated six times. In each replicate new plants were used and the position of the infected and uninfected plants were randomized.

2.6. *Larval development*

2.6.1. *On B. cinerea cultured on potato-dextrose agar*

Thirty Petrie plates (9 cm diameter) of PDA were inoculated using a 4 mm² PDA block from stock culture of *B. cinerea*. These plates were incubated at 21°C, 12L:12D. Thirty sterile PDA plates were used as control (Figure 4.3). After seven days, ensuring that *B. cinerea* was growing in the newly introduced plates, the 4 mm² PDA block with *B. cinerea* culture was removed. Two neonate larvae (<4 h after emergence) from sterile source were placed in every inoculated and sterile PDA
plates, which were sealed immediately using Parafilm. The plates were then incubated at 21°C, 12L: 12D. The plates were changed and frass was removed every six days retaining them in the sterile environment.

2.6.2. *On B. cinerea cultured on Murashige and Skoog medium*

An identical procedure (described in the above section 2.6.1) was applied to raise the larvae of *E. postvittana* on aseptically cultured *B. cinerea* on M–S medium (Murashige & Skoog, 1972). All procedures were conducted in the clean-air environment of the horizontal laminar-airflow bench (HWS120, Clyde–Apac, Sydney, Australia).

2.6.3. *On B. cinerea-infected and uninfected leaves of V. vinifera maintained on Knop’s solution*

From freshly obtained branches of glasshouse-raised *V. vinifera*, several 20-cm stem segments bearing 5–6 fully unfurled leaves were excised for use in this experiment. The stem segments were surface-sterilized using 1% NaClO solution for 5 min and rinsed in sterile water (3x). The leaves on these excised stem segments were then sprayed with the spore suspension in a laminar-airflow cabinet. The leaves on stem segments used as control were sprayed with sterile water. The basal tip of each segment was fixed on an Oasis® floral foam (4 cm³) soaked in Knop’s solution. The inoculated and uninoculated leaf-bearing stem segments were placed in a zip-lock plastic bag (35x40 cm²), which included a damp-filter paper. The set up was maintained at 22°C and 12L: 12D for 4–5 d.

Only those leaves bearing around 10% infection level were used in this experiment. The stem segments bearing either infected or uninfected leaves were placed separately in glass jars (22 cm tall, 15 cm diameter) (Figure 4.4). Two neonate larvae (<4 h after emergence) were placed on infected and uninfected leaves and maintained at 21±1°C, 60–70% RH, and 16L: 8D. After every four days, the stem segments were changed in order to maintain the infection level under 30% of leaf
Figure 4.1 Custom-made aluminium cage made for the free-choice experiment of adults of *Epiphyas postvittana* (not to scale) (am: aluminium mesh, i: infected plant, u: uninfected plant).

Figure 4.2 Custom-made glass device for the two-choice experiment using adults of *Epiphyas postvittana* (not to scale) (cp: circular port, gj: glass jar, hgt: horizontal glass tube, ip: infected plant, m: moth, p: Parafilm, up: uninfected plant)

Figure 4.3 Petri-dish pair for the development of the larvae of *E. postvittana* on *B. cinerea*. (not to scale) (l: larva, pd: Petri dish)
area and frass was removed from the plate. Fifty larvae were used for each treatment. Data pertaining to the rate of survival of the larvae were collected every four days. The dates of pupation, number of pupae, and pupal mass were recorded. Pupal mass was used as an indicator of adult-body mass, because adult body mass cannot be obtained without killing them. Each pupa was quarantined to a stoppered glass vial (4 cm tall, 2 cm diameter) until emergence. Adult emergence and sex ratio (%) were recorded. The adults were sexed and paired in a corrugated-wall Dixie cup enabling mating and oviposition. The adults enabled to mate and oviposit were subsequently measured for their performance: fecundity rate, possible delays in oviposition (measured in days), viability (measured as the number of emerged larvae). The eggs were incubated at 21±1°C, 60–70% RH, and 16L: 8D for 15 d enabling larval emergence. The number of days from the date of emergence of adults to death was also recorded.

2.6.4. On B. cinerea-infected and uninfected leaves of V. vinifera maintained on potato-dextrose agar or Murashige and Skoog medium

Excised leaves (petioles+laminae) of V. vinifera were surface-sterilized with 2% NaClO solution for 10 min and washed with double-sterile water (3x). After sterilization, each leaf was fixed onto either the PDA or M–S medium in a test tube (10 cm long, 1.5 cm diameter, sealed with Parafilm) by pushing the petiole through the solid medium (Figure 4.5). Leaves were sprayed with spore suspension, whereas sterile water sprays were used in control. All work was done on laminar-airflow bench, maintained at 22°C and 12L: 12D.

Leaves with 10% infection were used for rearing the larvae of E. postvittana. Two larvae were released on each leaf. Every four days, the survival larvae were noted and larvae were shifted onto either a new infected or a new uninfected leaf set up in the PDA medium as described above. The larvae were allowed to pupate and the life-history traits were noted. Fifty larvae were used for each treatment. Aseptic culture system has provided an environment to experimental verify the life-history
Figure 4.4 Custom-made glass device for the rearing of the larvae of *Epiphyas postvittana* (not to scale) (ff: floral foam, il: infected leaf, l: larva, ps: parafilm seal, ul: uninfected leaf)

Figure 4.5 Custom-made glass device for the rearing of the larvae of *Epiphyas postvittana* (not to scale) (l: larva, gt: glass tube, il: infected leaf, ms: medium slant, ps: Parafilm seal, ul: uninfected leaf)
performance of many plant feeding insects. One of the offshoots of such trials is raising the plant-feeding arthropods on their host plants in control environment (e.g., Forneck et al., 1999).

2.7. Statistical analysis

In the oviposition bioassay, the numbers of eggs laid on B. cinerea-infected and uninfected leaves in free-choice experiment and two-choice experiment were analyzed using paired sample ‘t’ test. Power function was applied to discriminate the significant difference in the mortality rate of larvae applying ‘y=a+bx’, where x is the day and y is the mortality. Non linear regression was used to fit the power function. An inverse exponential curve was fitted as expected, the rate of mortality to decrease with increasing time. A contingency table (χ²) was used to analyze female sex ratio, survival percentage of larvae from egg hatching to pupae, and survival percentage of pupae from pupation to adult emergence. Independent sample t-test was used to analyse all other life-history traits. Analyses were made with SPSS v17.0 (1993–2007) and GenStat (VSN International 2012). Graphs were generated in MS Excel 2013 and GenStat.

3. RESULTS

3.1. Oviposition behaviour

3.1.1. Free-choice experiment

Epiphyas postvittana females deposited significantly more number of eggs on uninfected leaves than on B. cinerea-infected leaves (uninfected vs infected, 732.6 vs 236, t= 13.91, p<0.001, Figure 4.6).

3.1.2. Two-choice Experiment

Gravid females of E. postvittana showed a significant preference for uninfected leaves (73.7%) as against the infected leaves (26.3%, n=30, χ²=6.53, p<0.05, Figure 4.7). The B. cinerea-infected leaves significantly inhibited oviposition, compared
with the uninfected leaves (uninfected vs infected, 399 vs 82, t= 4.36, p<0.01, Figure 4.8).

3.2. Larval development

3.2.1. On B. cinerea cultured on potato-dextrose agar

The mortality rate of the larvae of E. postvittana fed on B. cinerea on PDA (y=120.6-120.4*0.89^x) was significantly (p=0.039) lesser than the larvae that were fed only on PDA (y=106.61-107.57*0.78^x), where x represented time (days) and y represented the mortality rate (Figure 4.9). All larvae were dead at conclusion (15 d).

3.2.2. On B. cinerea cultured on Murashige and Skoog medium

The mortality rate of the larvae of E. postvittana fed on B. cinerea cultured (y=119.6-119.5*0.87^x) was significantly (p= 0.027) fewer than the larvae fed on only M–S medium (y=102.69-102.94*0.71^x, where x = day and y = mortality rate (Figure 4.10). All larvae were dead at conclusion (15 d).

3.2.3. On B. cinerea-infected and uninfected leaves of V. vinifera maintained on Knop’s solution

Larvae reared on B. cinerea-infected leaves developed faster than larvae reared on uninfected leaves of V. vinifera (Table 4.1). The mortality rate of larvae fed on B. cinerea-infected leaves (y=29.68-30.55*0.92^x) was not significantly different than those reared on uninfected leaves of V. vinifera (y=19.65-19.65*0.86^x), where x = day and y = mortality rate (Figure 4.11). Pupal masses of female moths reared on B. cinerea-infected leaves of V. vinifera were significantly greater (p<0.05) than larvae reared on uninfected leaves (Table 4.2). Botrytis cinerea infection did not affect pupation and adult emergence percentage, sex ratio of adult and the length of pre-oviposition period (Table 4.3). Botrytis cinerea-infected diet significantly increased
the fecundity rates \( p<0.05 \) and viability of eggs \( p<0.001 \) compared with uninfected leaves (Table 4.3).

3.2.3. *On B. cinerea-infected and uninfected leaves of V. vinifera maintained on potato-dextrose agar*

The mortality rate of larvae fed on *B. cinerea*-infected leaves \( y=27.19-27.26*0.96^x \) was not significantly \( p=0.925 \) different than those reared on uninfected leaves of *V. vinifera* \( y=21.70-21.91*0.94^x \), where \( x = \text{day} \) and \( y = \text{mortality rate} \) (Figure 4.12). Female larvae reared on *B. cinerea*-infected leaves developed faster than larvae reared on uninfected leaves of *V. vinifera* (Table 4.4). Pupal masses of female moths reared on *B. cinerea*-infected leaves of *V. vinifera* were significantly greater \( p=0.02 \) than larvae reared on uninfected leaves (Table 4.5). *Botrytis cinerea* infection did not affect the Pupation and adult emergence percentage, sex ratio of adult and the length of pre-oviposition period (Tables 4.5 and 4.6). *Botrytis cinerea*-infected diet significantly increased the fecundity rates \( p=0.02 \) and viability of eggs \( p=0.008 \) compared with control diet (Table 4.6).

3.2.4. *On B. cinerea-infected and uninfected leaves of V. vinifera maintained on Murashige and Skoog medium*

The mortality rate of larvae fed on *B. cinerea*-infected leaves \( y=22.55-23.05*0.94^x \) was not significantly \( p = 0.178 \) different than those reared on uninfected leaves of *V. vinifera* \( y=21.47-22.48*0.91^x \), where \( x = \text{day} \) and \( y = \text{mortality rate} \) (Figure 4.13). Larvae reared on *B. cinerea*-infected leaves developed faster than larvae raised on uninfected leaves of *V. vinifera* (Table 4.7). Pupal masses of female moths reared on *B. cinerea*-infected leaves of *V. vinifera* were significantly greater \( p=0.02 \) than larvae reared on uninfected leaves (Table 4.8). *Botrytis cinerea* infection did not affect the Pupation and adult emergence percentage, sex ratio of adult and the length of pre-oviposition period (Tables 4.8 and 4.9). *Botrytis cinerea*-infected diet significantly increased the fecundity rates \( p=0.03 \) and viability of eggs \( p=0.01 \) compared with control diet (Table 4.9).
Figure 4.6 Mean number of eggs laid by *E. postvittana* in a ‘free-choice’ experiment with uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera*.

Figure 4.7 Response of females *E. postvittana* in a ‘two-choice’ experiment towards uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera*.

Figure 4.8 Mean number of eggs laid by *E. postvittana* in a ‘two-choice’ experiment with uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera*.
Figure 4.9 Mortality rate of *E. postvittana* larvae reared solely on *B. cinerea* (■) and control (or PDA, □)

Figure 4.10 Mortality rate of larvae reared on *B. cinerea* (■) and control (or M−S medium, □)

Figure 4.11 Mortality rate of larvae reared on *B. cinerea*-leaves (■) or uninfected (□) leaves of *V. vinifera* using Knop’s solution
Table 4.1 Larval developmental time, pupal duration and adult life span (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using Knop’s solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval developmental time (d)</th>
<th>Pupal duration (d)</th>
<th>Adult life span(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Uninfected leaves</td>
<td>32.5±0.9</td>
<td>34.0±0.8</td>
<td>10.2±0.3</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>27.9±0.5</td>
<td>30.9±0.5</td>
<td>10.0±0.2</td>
</tr>
<tr>
<td>Statistic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.047</td>
<td>0.007</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*t = Independent sample t-test

Table 4.2 Pupal mass, percentage of larvae surviving from hatching to pupae, percentage of larvae surviving from hatching to adult and female sex ratio (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using Knop’s solution:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pupal mass (mg)</th>
<th>% surviving from hatching to pupa</th>
<th>% surviving from hatching to adult</th>
<th>Female sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Uninfected leaves</td>
<td>20.9±0.7</td>
<td>27.5±1.1</td>
<td>78</td>
<td>66</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>19.7±0.7</td>
<td>30.6±0.9</td>
<td>76</td>
<td>71</td>
</tr>
<tr>
<td>Statistic</td>
<td>t = 1.1</td>
<td>t = 2.1</td>
<td>χ² = 0.11</td>
<td>χ² = 0.58</td>
</tr>
<tr>
<td>p</td>
<td>0.25</td>
<td>0.04</td>
<td>0.737</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*t = Independent sample t-test

χ² = Contingency table (χ²)

Table 4.3 Adult performance (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera* using Knop’s solution:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Delay in egg laying (days)</th>
<th>Fecundity (no. eggs per female)</th>
<th>No. of larvae emerged per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected leaves</td>
<td>2.3±0.1</td>
<td>80.0±10.9</td>
<td>67.2±5.8</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>2.1±0.1</td>
<td>115.1±13.2</td>
<td>106.7±12.3</td>
</tr>
<tr>
<td>Statistic</td>
<td>t = 0.98</td>
<td>t = 2.5</td>
<td>t = 2.98</td>
</tr>
<tr>
<td>p</td>
<td>0.86</td>
<td>0.01</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*t = Independent sample t-test

χ² = Contingency table (χ²)
Figure 4.12 Mortality rate of larvae reared on *B. cinerea*-leaves (■) or uninfected (□) leaves of *V. vinifera* using PDA

Table 4.4 Larval developmental time, pupal duration and adult life span (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using PDA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval developmental time (d)</th>
<th>Pupal duration (d)</th>
<th>Adult life span (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Uninfected leaves</td>
<td>31.0±1.0</td>
<td>33.5±0.9</td>
<td>10.1±0.2</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected</td>
<td>29.7±0.8</td>
<td>30.3±0.6</td>
<td>9.7±0.2</td>
</tr>
<tr>
<td>leaves</td>
<td>Statistic</td>
<td><em>p</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 1.0</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 2.4</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 1.1</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 1.2</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 1.6</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>t</em> = 1.0</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

*p* = Independent sample *t*-test
Table 4.5 Pupal mass, percentage of larvae surviving from hatching to pupae, percentage of larvae surviving from hatching to adult and female sex ratio (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using PDA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pupal mass (mg)</th>
<th>% surviving from hatching to pupa</th>
<th>% surviving from hatching to adult</th>
<th>Female sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected leaves</td>
<td>Male 17.7±1.2</td>
<td>Female 25.1±1.1</td>
<td>80.9</td>
<td>70</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>18.3±0.5</td>
<td>28.5±1.1</td>
<td>82.3</td>
<td>76</td>
</tr>
<tr>
<td>Statistic</td>
<td>t = 0.5</td>
<td>t = 2.3</td>
<td>$\chi^2$ = 0.07</td>
<td>$\chi^2$ = 0.91</td>
</tr>
<tr>
<td>p</td>
<td>0.58</td>
<td>0.79</td>
<td>0.34</td>
<td>0.47</td>
</tr>
</tbody>
</table>

$t = $ Independent sample t-test

$\chi^2 = $ Contingency table ($\chi^2$)

---

Table 4.6 Adult performance (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera* using PDA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Delay in egg laying (days)</th>
<th>Fecundity (no. eggs per female)</th>
<th>No. of larvae emerged per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected leaves</td>
<td>2.1±0.1</td>
<td>95.6±12.0</td>
<td>84.3±10.8</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>2.0±0.1</td>
<td>130.4±7.9</td>
<td>120.6±10.4</td>
</tr>
<tr>
<td>Statistic</td>
<td>t = 1.0</td>
<td>t = 2.4</td>
<td>t = 3.0</td>
</tr>
<tr>
<td>p</td>
<td>0.33</td>
<td>0.02</td>
<td>0.008</td>
</tr>
</tbody>
</table>

$t = $ Independent sample t-test

$\chi^2 = $ Contingency table ($\chi^2$)
Figure 4.13 Mortality rate of larvae reared on *B. cinerea*-leaves (■) or uninfected leaves (◆) of *V. vinifera* using M–S medium

Table 4.7 Larval developmental time, pupal duration and adult life span (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using M–S medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval developmental time (d)</th>
<th>Pupal duration (d)</th>
<th>Adult life span (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Uninfected leaves</td>
<td>31.7±1.1</td>
<td>33.8±0.7</td>
<td>9.9±0.1</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected leaves</td>
<td>28.7±0.9</td>
<td>30.5±0.9</td>
<td>9.7±0.1</td>
</tr>
<tr>
<td>Statistic</td>
<td>t = 2.1</td>
<td>t = 2.2</td>
<td>t = 1.0</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.04</td>
<td>0.03</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*t* = Independent sample t-test
Table 4.8 Pupal mass, percentage of larvae surviving from hatching to pupae, percentage of larvae surviving from hatching to adult and female sex ratio (mean ± s.e) of E. postvittana reared on B. cinerea-infected or uninfected leaves of V. vinifera using M−S medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pupal mass (mg)</th>
<th>% surviving from hatching to pupa</th>
<th>% surviving from hatching to adult</th>
<th>Female sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected leaves</td>
<td>19.5±1.7</td>
<td>27.1±0.9</td>
<td>79.8</td>
<td>72</td>
</tr>
<tr>
<td>B. cinerea Infected leaves</td>
<td>20.9±0.9</td>
<td>30.5±1.1</td>
<td>79.2</td>
<td>76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic</th>
<th>t = 1.5</th>
<th>t = 2.3</th>
<th>( \chi^2 = 0.01 )</th>
<th>( \chi^2 = 0.42 )</th>
<th>( \chi^2 = 0.08 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.14</td>
<td>0.02</td>
<td>0.91</td>
<td>0.52</td>
<td>0.77</td>
</tr>
</tbody>
</table>

\( t \) = Independent sample t-test  
\( \chi^2 \) = Contingency table (\( \chi^2 \))

Table 4.9 Adult performance (mean ± s.e) of E. postvittana reared on B. cinerea-infected and uninfected leaves of V. vinifera using M−S medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Delay in egg laying (days)</th>
<th>Fecundity (no. eggs per female)</th>
<th>No. of larvae emerged per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected leaves</td>
<td>2.1±0.1</td>
<td>90.2±10.8</td>
<td>74.3±8.8</td>
</tr>
<tr>
<td>B. cinerea Infected leaves</td>
<td>2.2±0.1</td>
<td>122.4±9.9</td>
<td>103.9±9.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic</th>
<th>t = 0.98</th>
<th>t = 2.2</th>
<th>t = 2.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.86</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\( t \) = Independent sample t-test  
\( \chi^2 \) = Contingency table (\( \chi^2 \))
4. DISCUSSION

Gravid *E. postvittana* prefer to oviposit on uninfected leaves as against that of *B. cinerea*-infected leaves of *V. vinifera*. The larvae of *E. postvittana* feed on *B. cinerea*-infected leaves develop quicker, attain a heavier pupal mass particularly in those developing as females, and the adults lay more numbers of eggs than those raised on uninfected leaves of *V. vinifera*. The viability rate of eggs hatching to neonates too is greater in those adults reared on *B. cinerea*-infected leaves.

4.1. Females of *E. postvittana* do not prefer ovipositing on *B. cinerea*-infected leaves of *V. vinifera*

In the present study, the gravid individuals of *E. postvittana* were deterred during oviposition by the olfactory cues arising from *V. vinifera* leaves infected by *B. cinerea*. This observation concurs with my previous findings that individuals of gravid *E. postvittana* are deterred from oviposition when offered *B. cinerea*-infected berries of *V. vinifera* (Rizvi *et al.*, 2015b). Preference for oviposition in different species of Lepidoptera has been shown to be regulated generally by either stimulatory or inhibitory cues arising from host plants (Renwick, 1989). Fungus infection can modify the volatiles arising from a plant by interfering with the plant’s biosynthetic pathways.

Volatile, arising during pathogenic interactions between plants and fungi, act either as attractants (Cossé *et al.*, 1994; Mondy *et al.*, 1998; Cardoza *et al.*, 2003) or deterrents (Döterl *et al.*, 2009; Tasin *et al.*, 2011) for insects in three-way, three-component interacting systems. Infection by *B. cinerea* on *V. vinifera* alters its volatile complexion either by changing their quantity or by producing new compounds, such as alcohols, terpenes, and benzoates (Tasin *et al.*, 2012). *Botrytis cinerea*-infected berries of *V. vinifera* include several new alcohols (Tasin *et al.*, 2012), which deter *L. botrana*. Receptors on the dendritic membrane of sensory neurons of *E. postvittana* and their specific receptivity reiterate that *E. postvittana* is capable of recognizing a range of volatiles (e.g., alcohols, terpenoids and benzoates) and volatile-stress signals such as methyl salicylate (Jordan *et al.*, 2009).
A gravid Lepidoptera usually prefers, for oviposition, those sites which maximize its opportunities for a better performance of her progeny (Jaenike, 1978). This builds on the premise that juvenile-life stages have a limited capacity to move on host surfaces and therefore the gravid female chooses the best possible site for oviposition guaranteeing reasonable nutrition to her offspring. In contrast, several arguments and explanations prevail indicating that adult nutrition influences oviposition decision (Mayhew, 1997; Scheirs & De Bruyn, 2002). For example, *Chromatomyia nigra* (Diptera: Agromyzidae) and *Altica carduorum* (Coleoptera: Chrysomelidae) have been demonstrated to select plants for oviposition that maximize their own fitness rather than that of their progeny, indicating that optimum foraging determines oviposition decisions (Scheir *et al*., 2000; Scheirs & De Bruyn, 2002). In *E. postvittana*, the oviposition preference is essentially governed by plant-surface cues, since it does not lay eggs on rough and hairy surfaces (Tomkins *et al*., 1991; Foster & Howard, 1999). Incidence of fungal mycelia on the leaf surfaces of *V. vinifera*, mimicking hairiness precludes oviposition (Rizvi *et al*., 2015a, b). The lepidopteran larvae are highly mobile and voracious feeders and, therefore, do not connect strongly with Jaenike’s (1978) hypothesis that explains mother’s choice of site for oviposition benefitting the offspring (Roitberg & Mangel, 1993; Suckling & Brockerhoff, 2010). Under experimental conditions, the larvae of *E. postvittana* have been established as highly active feeders on plants of unrelated and various taxa and gravid females lay eggs on those ranges of plants (Foster & Howard, 1999).

**4.2. Botrytis cinerea-infected leaves positively influence larval performance of E. postvittana**

Insect and fungus association ranges from non-specific polyphagy to obligate mutualism (Jonsell & Nordlander, 2004). In the present study, oviposition choices made by adults of *E. postvittana* do not synchronize with the best performance of the larvae. This mismatch could be because either the larvae and adults of *E. postvittana* have differing host requirements or these life forms feed on different organs of different plants. The larvae of *E. postvittana* feed on leaves and fruits of *V. vinifera*, *M. domestica*, and different species of *Citrus*; whereas their adults feed on floral nectar. My results suggest a mutualistic relationship between the larvae of *E.
postvittana and B. cinerea. The larvae develop more rapidly, attain greater pupal mass (particularly in the females), and lay more eggs when fed on B. cinerea-infected leaves. Larval diet plays an important role in determining the size and reproductive performance in the Lepidoptera (Mondy et al., 1998), where the adult females utilize the energy stored by the larvae for egg production (Boggs, 1997). Botrytis cinerea plays an essential role in the larval diet and the larvae of L. botrana have been demonstrated to disperse the conidia of B. cinerea to adjacent berries of V. vinifera (Mondy & Corio-Costet, 2000). Further, the injury caused by the larvae of L. botrana while feeding on V. vinifera tissues facilitates the colonization and establishment of B. cinerea (Fermaud & Le Menn, 1992). Cardoza et al., (2002) found that leaves of A. hypogaea infected with a necrotrophic fungus S. rolfsii preferably fed by S. exigua and had a positive effect on key life-history traits when fed on S. rolfsii-infected A. hypogaea.

Botrytis cinerea is an opportunistic necrotrophic fungus, which, under favourable conditions can kill its hosts. The level and nature of infection by fungi can influence the oviposition behaviour of insects (Biere & Tack, 2013). It is, therefore, possible that over time, the effect of B. cinerea can alter the behaviour of E. postvittana. Nonetheless, the caveat here is that the levels of fungal infection used in the present study have indeed been low inducing a sub-lethal infection on V. vinifera leaves. In such a context, the choice of gravid females in avoiding B. cinerea-infected leaves of V. vinifera could be seen as an adaptive strategy because the difference between the time of oviposition and that of egg hatch, which potentially enhances the infection levels on V. vinifera leaves. Therefore, the larval-food quality could have deteriorated with accelerated level of infection or the production of a defensive compound could have reached up to the lethal level (Tasin, 2012). This explains why B. cinerea-infected leaves are unattractive for the gravid females for oviposition.

4.3. The larvae of E. postvittana cannot survive and develop by feeding only on B. cinerea

Lepidopteran larvae require high nutritional levels to match their rapid growth before pupation (Slansky & Scriber, 1985). For example, the host-plant quality has been
shown to have a significant, indirect but positive effect on pupation in *Manduca sexta* (Lepidoptera: Sphingidae) through accelerated growth during its larval stages (Diamond & Kingsolver, 2011). Physiological changes in the larvae in preparation for pupation indicate that later instars switch from protein-based diets to lipid-based diets to accumulate more of membrane-bound energy (Stockhoff, 1993; Ojeda–Avila *et al*., 2003). Insects are generally shown to lack the capacity to synthesize sterols that are necessary as precursors to steroids which act as growth regulators. The insects, therefore, acquire sterols from their host plants and/or from associated symbionts (Nes *et al*., 1997; Behmer & Nes, 2003). Fungi are an excellent source of sterols and vitamins, which are vital in insect development (Chapman *et al*., 2012). Lipids, as principal sources of stored energy and as precursors of steroids are key for insects particularly during metamorphosis (Arrese & Soulages, 2010). Nevertheless, high levels of lipids and low levels of other essential nutrients appear to stress insects by affecting metabolism and inducing an inability to maintain homeostasis (Lord, 2010). One possible reason for the death of larvae reared on exclusive fungus diet in my study could be that the larvae of *E. postvittana* did not get sufficient energy-containing nutrients, such as carbohydrates and proteins from *B. cinerea*, although they may have had a rich supply of lipids from *B. cinerea*. A second possibility is that the concentrated dose of lipidic materials may have induced a dietary imbalance as shown in the larvae of *M. sexta* parasitized by *Cotesia congregata* (Hymenoptera: Braconidae) (Thompson & Redak, 2008).

5. CONCLUSION

In conclusion, gravid females of *E. postvittana* use olfactory and volatile cues to ‘evaluate’ infection levels on *V. vinifera* caused by *B. cinerea* to oviposit. Larvae of *E. postvittana* show a mutualistic relationship with *B. cinerea*. On *B. cinerea*–infected *V. vinifera* leaves, the larvae have a shorter larval duration, attain heavier pupal mass and the emergent adults lay more eggs than the larvae fed on uninfected *V. vinifera* leaves. The larvae reared on exclusive-fungus diet died in 15 d indicating that for a better larval performance and oviposition rate of *E. postvittana*, the *V. vinifera*—*B. cinerea* interacting system is but imperative. Further chemical-
ecological verifications are necessary to establish the role of each participant in the life-history performance of *E. postvitanna*.

### 6. REFERENCES


Chapter 5

*Botrytis cinerea* induced changes in *Vitis vinifera* leaves influence the oviposition behaviour and life history of *Epiphyas postvittana**

*Submitted paper (details below) presented with minor modifications.
1. INTRODUCTION

Among plant-feeding insects, right selection of plants by gravid females is critical for offspring performance. Suitable plants influence the outcomes such as egg numbers and quality, further to playing a key role in the growth, development, and fecundity (Awmack & Leather, 2002). The link between choice of the plant made by the gravid female on the one hand and the progeny’s survival and development on the other dictates the context for preference–performance hypothesis (Jaenike, 1978). In an evolutionary perspective, the gravid female selects a site that will subserve the nutritional needs of her offspring, because in most instances the neonates would have limited opportunities to explore new, potential nutrition sites (Mayhew, 1997). The preference–performance hypothesis reiterates that the preferred site for oviposition should meet the nutritional needs of the offspring.

Adult females maximize their offspring fitness by ovipositing at appropriate sites on the plant. Availability and suitability of plants vary in space and time because of the heterogeneous environment. The gravid females usually show a preference to a particular plant or a site for oviposition among various plants in a natural environment, yet that oviposition preference needs to synchronize with the performance of the offspring is currently being debated (Nyman et al., 2011).

Locating right plants is critical for plant-feeding insects to meet their nutritional requirements and also for suitable sites for oviposition. Successful location of the plant by the insect necessitates recognition of physical and chemical cues in a complex environment comprising varied trigger factors. Plant volatiles play a key role in such host recognition by plant-feeding insects. The dietary breadth of polyphagous insects influences the degree to which plant volatiles are utilized in recognition (Vet & Dicke, 1992). Recognition via olfactory signals occurs by using either species-specific compounds or specific ratios of commonly occurring compounds (Bruce et al., 2005). Among plant-feeding insects, the preference for certain plants, the decision to lay eggs or not, and the number of eggs laid are determined by the cues from the plants (Renwick & Chew, 1994). Olfaction, contact chemoreception, and vision enable this. Nevertheless, olfactory cues from plants orientate gravid females to potential oviposition sites over a distance (Bruce et al.,
The sensory cues of a plant-feeding insect enable decision making on the appropriateness of a plant (Gripenberg et al., 2010).

In the natural environment, various organisms attack plants. Among these, insects and fungi, which attack plant simultaneously, establish a three-way interacting system. In such contexts, the fungi influence the interaction between the insect and plant, which can be either mutualistic or antagonistic, and, occasionally neutral as well (Hatcher, 1995). Fungi induce variations in the plant chemistry and consequently alter plant-volatile profiles (Cardoza, 2003b; Raman et al., 2012). Such induced variations in plant volatiles are recognized by the olfactory receptors of Lepidoptera as either an attractant (Cardoza et al., 2003b) or a deterrent (Tasin et al., 2012). *Botrytis cinerea* is known to degrade hydrocarbons and release volatiles, such as 3-methyl-1-butanol — an indicator of tissue rot in plants (Tasin et al., 2012). Such changes occurring sequel to tissue rot and the consequently released volatiles are recognized by gravid *Lobesia botrana* (Lepidoptera: Tortricidae) repudiating it from oviposition (Tasin et al., 2012). Fungal infection of a plant can suppress the plant’s defence capability against plant-feeding insects by modifying the plant’s secondary metabolism and the nutritional quality of the plant as well, thus rendering it amenable for insect colonization (Cardoza et al., 2003a). On the other hand, fungal infection can deteriorate the nutritional quality of the plant and influence plant-feeding insect’s development negatively (Tasin et al., 2012).

In Australian vineyards, gravid females of *Epiphyas postvittana* (Lepidoptera: Tortricidae) commonly encounter the pathogenic fungus *B. cinerea*, particularly during oviposition (Chapter 1). Adults of *E. postvittana* do not generally display any notable level of attraction towards *B. cinerea*-infected leaves of *V. vinifera*, although under ‘no-choice’ trials they lay few eggs on *B. cinerea*-infected *V. vinifera* (Rizvi et al., 2015a). On the contrary, *E. postvittana* larvae when allowed to feed on either *B. cinerea*-infected berries or mildly infected leaves of *V. vinifera*, they show an improved life-history performance (Rizvi et al., 2015a, b). Incidentally they also disperse *B. cinerea* conidia mechanically (Lo & Murrell, 2000).

My earlier studies indicate that the infection status of *B. cinerea* alters the preference behaviour of *E. postvittana*: the larvae of *E. postvittana* prefer ‘mildly’
and ‘moderately’ infected leaves, but never the ‘intensely’ infected leaves of *V. vinifera* (Rizvi *et al.*, 2015a). I, therefore, hypothesized that gravid females of *E. postvittana* discriminate between *B. cinerea*-infected and uninfected leaves of *V. vinifera* for oviposition, thus maximizing their offspring performance. This was verified by characterizing volatiles in *B. cinerea*-infected and uninfected leaves of *V. vinifera* of comparable age. The selection behaviour of gravid females of *E. postvittana* in discriminating infected against uninfected leaves of *V. vinifera* was assayed in a wind tunnel. The larval performance was determined by enabling the larvae to live and feed on infected and uninfected leaves of *V. vinifera*. In the above context, I sought answers to the following questions: Does the infection by *B. cinerea* alter the volatile profile of *V. vinifera*? Does the infection by *B. cinerea* on *V. vinifera* attract gravid females of *E. postvittana*? Does the infection of *B. cinerea* on *V. vinifera* influence the survival rate and the larval performance of *E. postvittana*?

2. **MATERIAL AND METHODS**

2.1. **Insect rearing**

Eggs of *E. postvittana* were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O). The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm$^3$) following the method described in Chapter 2). The cultures were maintained at 21±1°C, 60–80% RH, and 16L: 8D regimen. All tests were performed under identical environmental conditions. Only those females, which laid 5–10 eggs, were used in the oviposition bioassays. Adult males and females were not exposed to either uninfected or *B. cinerea*-infected leaves of *V. vinifera*, prior to experimentation.

2.2. **Fungus culture and preparation of conidial suspension**

The conidial suspension was prepared in sterile water following the method described in Chapter 3) and adjusted to $10^6$ conidia/ml.
2.3. **Inoculation of *V. vinifera* leaves with *B. cinerea***

Fifty plants of *V. vinifera* were raised in plastic pots in a same way as described in Chapter 4. Plants, 7–8 wk old, were used. Plants were inoculated with *B. cinerea* by following the method described in Chapter 5.

Because the larvae of *E. postvittana* showed a better life-history performance when fed on mildly infected (10–30%) leaves of *V. vinifera* with *B. cinerea* (Rizvi *et al.*, 2015a), in this study I used infection level 30–60% to determine the volatiles from *V. vinifera* leaves. Beyond 70% infection level, the leaves abscised from the stem in natural conditions (Rizvi, 2014, unpublished observations).

2.4. **Headspace collection and volatile profiling**

Volatile emanating from either uninfected or infected leaves were collected using solid-phase microextraction (SPME) technique. The upper rim of the pot (that included either an uninfected or a *B. cinerea*-infected *V. vinifera* shoots) was covered with aluminium foil (CLOROX Commercial Padstow, NSW, Australia) and overlaid with Parafilm®. The infected or uninfected shoots were enclosed in glass bell jars (LUREX flanged bell jar with a top cork hole; 50 cm tall, 35 cm wide) for 60 min. The 5-cm wide cork hole was sealed with Parafilm (Figure 5.1). The SPME fibre coated with divinylbenzene–carboxen–polymethyl-siloxane (1 cm, 23 Ga, 50/30 µm film thickness, SUPELCO, Bellefonte, PA, USA) was preconditioned in the injector port of the gas chromatograph [GC] at 250°C for 5 min. Volatiles from the leaves were collected at room temperature (24–27°C) for 60 min using the preconditioned SPME fibre by inserting it through the Parafilm-sealed cork hole of the bell jar. At the end of 60 min, the fibre was instantly inserted into the injector port of the GC–MS (Agilent 7890A GC and 5975C MSD, Agilent, Palo Alto, CA, USA interfaced to a Gersal MPS 2XL autosampler) at 250°C, and the volatiles were assayed. The GC was equipped with a BP21 fused-silica capillary column (30 m x 0.25 mm [internal diameter], film thickness 0.25 µm; SGE Analytical Science, Melbourne, Australia), which was eluted with helium (He) flowing at the rate of 1.5 ml/min and temperature programmed to rise from 40°C (hold 3 min) to 180°C at the
rate of 4°C rise/min and to 220°C (hold time 5 min) at the rate of 10°C rise/min. The GC–MS interface temperature was set at 250°C. The MS operated in the electron-ionization mode (EI, internal ionization source; 70 eV) at the scan range 50–700 m/z. Six replicates each of uninfected and *B. cinerea* infected plants were analyzed for volatiles over a four day period. A blank SPME fibre was run after each sample collection to ensure that no contamination occurred on the SPME fibre.

2.4.1. **Identification of the compounds**

A majority of compounds was identified according to their mass spectra and retention times by comparing with authentic standards from Sigma–Aldrich (St. Louis, Missouri) and Merck KGaA (Darmstadt, Germany). Those compounds whose authentic standards were unaffordable were identified consulting NIST library (>80% probability) (NIST/EPA/NIH Mass Spectral Library, version 2.0g) and AnalyzerPro (V3.1.0.0 Build 2729) databases. The source (or the method) used to determine the identity of specific compounds is notified in results, *via* Table 5.1.

2.5. **Wind-tunnel assay**

Olfactory behaviour of *E. postvittana* towards *B. cinerea*-infected and uninfected leaves of *V. vinifera* was verified using a custom-made wind tunnel. A wind tunnel (Figure 5.2) consisting of a flight segment of 180 x 80 x 80 cm³ was constructed using plexiglass. A centrifugally spinning fan at the upwind end of the tunnel provided airflow into the tunnel and a centripetally spinning fan at the downwind end, spinning at the same velocity as that of the upwind-end fan, enabled air movement (Cha *et al.*, 2008). Short plastic sleeves secured the fans on either side to the tunnel. The perimeter of the sleeve encircling the tunnel was sealed using Scotch tape. Commercially marketed charcoal filters (WICKES Cooker Hood Charcoal Filter, Alton, England) (79.9 x 79.9 cm²) were fixed between the ends of the wind-tunnel and the two fans flanking on either side to ensure deodorization of air. A cheesecloth screen at the downwind end prevented escape of *E. postvittana* adults.
From the downwind end, on the outside of the tunnel, every 20 cm distance was marked using a permanent marker pen.

Air at a velocity of 25 cm/s was propelled at the upwind end of the wind tunnel. The pot was completely covered with Parafilm to prevent any gaseous material emitting from either the potting mixture or the pot. A *V. vinifera* plant in a pot (either infected or uninfected) serving as an odour stimulus was placed at 35 cm distance from the side edge and at 20 cm distance from the upwind end of the tunnel. Two hours before the end of the photophase (light phase of the photoperiod), gravid females of *E. postvittana* were placed in the downwind end of the tunnel in a 250 ml glass beaker covered by a pierced Parafilm sheet enabling aeration, to acclimate them to the tunnel environment. At scotophase (dark phase of photoperiod) the Parafilm sheet was removed enabling *E. postvittana* to fly freely in the tunnel for 30 min. *Epiphyas postvittana* behaviour was scored for (1) no activity (confined to the beaker), (2) activity (*E. postvittana* fly upwind <20 cm), (3) activity (*E. postvittana* fly upwind 20−60 cm), (4) activity (*E. postvittana* fly upwind 60−120 cm), and (5) activity (*E. postvittana* fly upwind 120−180 cm; 170 cm earmarked the odour source, viz., the uninfected or *B. cinerea*-infected plant). Ten runs (*n*=10) were made against either *B. cinerea*-infected or uninfected *V. vinifera*. At the conclusion of each test run, the beaker containing *E. postvittana* adults was moved around placed at different points along the breadth of the tunnel (80 cm).

2.6. Larval development

From freshly obtained shoots of glasshouse-raised *V. vinifera*, several 20-cm stem segments bearing 5−6 fully open leaves were excised for use in this experiment. The stem segments were surface-sterilized using 1% NaClO solution for 5 min and rinsed in sterile water (3x). The leaves on these excised stem segments were then sprayed with the spore suspension in a laminar-airflow cabinet. The leaves on stem segments used as control were sprayed with sterile water. The basal tip of each segment was fixed on an OASIS® floral foam (4 cm³) soaked in KNOP’s solution. The inoculated and uninoculated leaf-bearing stem segments were placed in a zip-
lock plastic bag (35x40 cm$^2$), which included a damp-filter paper. The set up was maintained at 22°C and 12L: 12D for 15–16 d.

Only those leaves with 60% infection level were used in this experiment. Two neonate larvae (<4 h after emergence) were placed on infected or uninfected leaves and maintained at 21±1°C, 60–70% RH, and 16L: 8D. After every four days, leaves were changed and frass was removed from the plate. Fifty larvae were used in each treatment. Data pertaining to the rate of survival of the larvae were collected every four days. The dates of pupation, number of pupae, and pupal mass were recorded. Pupal mass was used as an indicator of adult-body mass, because adult body mass cannot be obtained without killing them. Each pupa was quarantined to a stoppered glass vial (4 cm tall, 2 cm wide) until emergence. Adult emergence and sex ratio (%) were recorded. The adults were sexed and paired in a corrugated-wall Dixie cup enabling mating and oviposition. Adults enabled to mate and oviposit were subsequently measured for their performance: fecundity rate, possible delays in oviposition (measured in days), viability of eggs (measured as the number of emerged larvae). The eggs were incubated at 21±1°C, 60–70% RH, and 16L: 8D for 15 d enabling larval emergence. The number of days from the date of emergence of adults to death was also recorded.

2.7. Statistical analysis

A contingency table ($\chi^2$ test) followed by a Ryan Multi-comparison Test was applied to screen for significant differences in each behavioural step of adults of $E. postvittana$ in the wind-tunnel assay. Non-linear regression was applied to discriminate the significant difference in the mortality rate of the larvae between $B. cinerea$-infected and uninfected leaves of $V. vinifera$ using the equation $Y = A + Bx$, where $x$ is the time (d), $y$ is mortality and $A$, $B$ and $R$ (rate of curvature) are estimated parameters. An inverse exponential curve model was used because the expected rate of mortality decreased with increasing time. Analyses were conducted with GenStat (VSN International 2012) (Hertfordshire, UK). Table was generated in MS Excel 2013.
3. RESULTS

3.1. Volatile profile

Multiple volatile acids, alcohols, aldehydes, aromatics, hydrocarbons, ketones, and terpenes were detected from *B. cinerea*-infected and uninfected *V. vinifera* leaves: uninfected materials emitted 13 compounds, whereas *B. cinerea*-infected materials 23 (Table 5.1). The most abundant compound in the uninfected *V. vinifera*, measured by total integrated peak area of GC elution, was nonanal, whereas in the infected *V. vinifera* it was 2-hexen-1-ol (E). The other high-quantity compounds in uninfected leaves were benzaldehyde, acetic acid, hexanal, and 4,8-dimethyl-1,1,(E)3,7-nonatriene, whereas 2-hexenal (E), 1-hexanol, and 3-octanone were the most abundant compounds in infected leaves.

3.2. Wind-tunnel assay

The difference between flights of gravid females of *E. postvittana* towards uninfected and *B. cinerea*-infected leaves was significantly different. A significant inhibition in attraction was measured in females of *E. postvittana* towards *B. cinerea*-infected leaves compared to uninfected leaves of *V. vinifera* ($\chi^2=9.82$, $p=0.04$, Figure 5.3). Volatiles emanating from uninfected leaves attracted *E. postvittana* females significantly ($p<0.001$). Greater number of females (>70%) responded to uninfected *V. vinifera* positively than those responding (33%) to infected. Although only 12% of gravid females landed on the uninfected *V. vinifera* (covered the entire length of the wind-tunnel), the difference was significant ($p<0.05$) compared with the number of females (2%) landing on infected leaves.

3.3. Larval development

The larvae fed on uninfected leaves of *V. vinifera* completed their larval period and successfully pupated (Table 5.2 and 5.3). Adult females oviposited in 48 h of mating (Table 5.3), whereas, the larvae fed on *B. cinerea*-infected leaves of *V. vinifera* showed 100% mortality in 15 d. The mortality rate of larvae fed on *B. cinerea*-infected leaves ($y=115.1-122*0.92^x$, where $x$=day and $y$=mortality rate; Figure 5.4).
Figure 5.1 Custom-made device for volatile profiling from the leaves (not to scale) (al: aluminium foil, f: SPME fibre, bj: bell jar, il: infected leaf, p: pot, ps: parafilm seal, ul: uninfected leaf)

Figure 5.2 Custom-made wind tunnel to determine the olfactory behaviour of E. postvittana toward B. cinerea-infected and uninfected V. vinifera [A] Vertical-sectional view [B] Cross-sectional view (not to scale). cc—cheesecloth, cf—charcoal filter, cff—centrifugal fan (upwind end), cpf—centripetal fan (downwind end), il—infected leaves, m—moth, pp, pierced Parafilm, ps—plastic sleeve, sf—polyvinyl supporting frame, st—sticky tape, ul—uninfected leaves, ←—air direction.
Table 5.1 Volatile compounds (mean±s.e) (detection frequency) detected from Botrytis cinerea and uninfected leaves of V. vinifera via SPME. Bold faced type represents volatiles that significantly differed between B. cinerea-infected and uninfected plants (p<0.05)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative area in percentages</th>
<th>Source of laboratory standards*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected plant</td>
<td>Botrytis cinerea-infected plant</td>
</tr>
<tr>
<td>Hexanal</td>
<td>10.68±1.3 (6)</td>
<td>3.27±0.6 (6)</td>
</tr>
<tr>
<td>3-Octanone</td>
<td>-</td>
<td>9.04±1.4 (6)</td>
</tr>
<tr>
<td>1-Hexanol</td>
<td>-</td>
<td>10.72±1.9 (5)</td>
</tr>
<tr>
<td>2-Hexen-1-ol (E)</td>
<td>-</td>
<td>16.12±2.3 (6)</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>-</td>
<td>2.43±0.9 (4)</td>
</tr>
<tr>
<td>Limonene</td>
<td>2.0±0.6 (4)</td>
<td>1.41±0.2 (5)</td>
</tr>
<tr>
<td>Propyl benzene</td>
<td>1.41±0.3 (4)</td>
<td>2.3±0.2 (4)</td>
</tr>
<tr>
<td>2-Hexenal (E)</td>
<td>-</td>
<td>12.32±2.5 (6)</td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>-</td>
<td>3.87±0.9 (6)</td>
</tr>
<tr>
<td>4,8-dimethyl-1,(E)3,7-</td>
<td>7.39±3.2 (5)</td>
<td>5.21±0.9 (5)</td>
</tr>
<tr>
<td>nonatriene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>-</td>
<td>0.42±0.05 (6)</td>
</tr>
<tr>
<td>Nonanal</td>
<td>29.2±5.2 (6)</td>
<td>6.69±1.2 (6)</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>-</td>
<td>2.00±0.3 (6)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>11.13±1.3 (6)</td>
<td>2.07±0.56 (6)</td>
</tr>
<tr>
<td>2-Ethyl-1-hexanol</td>
<td>-</td>
<td>2.78±0.6 (5)</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>13.0±1.9 (6)</td>
<td>3.94±1.2 (6)</td>
</tr>
<tr>
<td>α-Farnesene</td>
<td>5.2±0.6 (5)</td>
<td>2.55±0.3 (5)</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>4.57±1.2 (3)</td>
<td>1.34±0.5 (3)</td>
</tr>
<tr>
<td>α−Terpineol</td>
<td>1.0±0.2 (5)</td>
<td>5.7±1.3 (6)</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0.56±0.06 (4)</td>
<td>0.66±0.1 (5)</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>-</td>
<td>3.32±0.8 (4)</td>
</tr>
<tr>
<td>Tridecane</td>
<td>1.5±0.4 (3)</td>
<td>0.18±0.02 (5)</td>
</tr>
<tr>
<td>Octadecane</td>
<td>2.1±0.9 (5)</td>
<td>1.02±0.1 (4)</td>
</tr>
</tbody>
</table>

* SA—Sigma-Aldrich; MK—Merck
The remainder have been determined following either NIST or AnalyzerPro databases
Figure 5.3: Olfactory response of gravid females of *Epiphyas postvittana* towards *Botrytis cinerea*-infected leaves (●) and uninfected leaves of *V. vinifera* (○) in the wind tunnel. Within each behavioural step, circle labelled with different letters are significantly different (p<0.05).

Figure 5.4 Mortality rate of larvae reared on *B. cinerea*-infected (●) or uninfected (○) *V. vinifera*
Table 5.2 Larval developmental time, pupal duration and adult life span (mean±s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval developmental time (d)</th>
<th>Pupal duration (d)</th>
<th>Adult life span(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.2±1.1</td>
<td>34.9±0.6</td>
<td>10.0±0.5</td>
<td>9.9±0.3</td>
</tr>
<tr>
<td>Infected</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5.3 Pupal mass, percentage of larvae surviving from hatching to pupae, percentage of larvae surviving from hatching to adult and female sex ratio (mean±s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pupal mass (mg)</th>
<th>% surviving from hatching to pupa</th>
<th>% surviving from hatching to adult</th>
<th>Female sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>19.5±1.1</td>
<td>82</td>
<td>70</td>
<td>51</td>
</tr>
<tr>
<td>Infected</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5.4 Adult performance (mean±s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Delay in egg laying (days)</th>
<th>Fecundity (no. eggs per female)</th>
<th>No. of larvae emerged per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>1.9±0.1</td>
<td>95.0±10.9</td>
<td>75.2±5.8</td>
</tr>
<tr>
<td>Infected</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Volatiles from *B. cinerea*-infected and uninfected *V. vinifera* determined the selection behaviour of gravid females of *E. postvittana*. Larval performance was determined by enabling the larvae to live and feed on infected (60%) and uninfected leaves of *V. vinifera*. The uninfected leaves of *V. vinifera* were significantly more (*p*<0.05) attractive to gravid females of *E. postvittana* and a better life-history performance of *E. postvittana* occurred in those raised on uninfected *V. vinifera* than infected leaves.

4.1. *Botrytis cinerea* infection alters the volatile profile of *V. vinifera*

My results demonstrate that fungal infection affects the quality and quantity of volatiles emanating from *V. vinifera*. I present here evidences that volatiles emitted by *B. cinerea*-infected *V. vinifera* differ qualitatively and quantitatively from those emitted by uninfected leaves of *V. vinifera*. Infection by pathogenic fungi alters the chemistry of plants and influences the volatile composition of them (Mondy & Corio-Costet, 2000; Dötterl *et al*., 2009). For example, infection of *Zea mays* by *Setosphaeria turcica* (Pleosporales: Pleosporaceae) suppresses the emission of *S. littoralis* induced plant volatiles such as (E)–β–farnesene, linalool, (3E–4,8–dimethyl–1,3,7–nonatriene, geranyl acetate, and indole (Rostás *et al*., 2006). On the other hand, *A. hypogaea* infected by *S. rolfsii* releases E–4,8–dimethyl–1,3,7–nonatriene, (E,E)–4,8,12–trimethyl–1,3,7,11–tridecatetraene, methyl salicylate, and 3–octanone, which, in turn, attract *S. exigua* (Cardoza *et al*., 2002). Volatile products from the lipoxygenase pathway are the green-leaf volatiles and these account for >50% of emissions from damaged plants (Holopainen, 2004). I identified five lipoxygenase products among the volatiles from *B. cinerea*-infected leaves of *V. vinifera*. The dominant lipoxygenase products were C6 compounds hexanal, 1–hexanol, 2–hexen–1–ol (E), 2–hexanal (E), and one C8 compound 1–octen–3–ol. These five compounds overall contributed to 45% of total volatiles emitted in *B. cinerea*-infected *V. vinifera*. A similar result has been reported by Jansen *et al*., (2009) that *Solanum lycopersicum* (Solanales: Solanaceae) infected by *B. cinerea* releases lipoxygenase volatile compounds, such as 3–hexenal, 3–hexenol
2-hexenal, 1-penten-3-ol, and 3-hexenyl-acetate. 1-octen-3-ol occurs ubiquitously in fungi, and is considered a characteristic element for the ‘fungal aroma’ (Combet et al., 2006). In addition to emission of lipoxygenase products, four terpenes — β-ocimene, limonene, α-farnesene, and terpineol — too were identified among volatiles arising from B. cinerea-infected V. vinifera leaves. Several terpenoids have distinct role in plant defence against biotic and abiotic stress factors (Singh & Sharma, 2015). An increase in the production of monoterpenes occurs with the increasing intensity of B. cinerea infection on S. lycopersicum (Jansen et al., 2009). I found 3-octanone from B. cinerea-infected V. vinifera leaves. 3-octanone characteristically arises from fungi-infected plants; for example, A. hypogaea releases 3-octanone when infected by S. rolfssii (Cardoza et al., 2002), Triticum aestivum and Avena sativa (Poales: Poaceae) release 3-octanone, when infected by either Penicillium glabrum or P. roqueforti (Eurotiales: Trichocomaceae) (Börjesson et al., 1992). Nonanal, hexanal, benzaldehyde, and acetic acid abundantly occur in uninfected V. vinifera leaves. In B. cinerea-infected leaves, nonanal, hexanal, benzaldehyde, and acetic acid occur in low quantities.

4.2. Infection by B. cinerea on V. vinifera does not attract gravid females of E. postvittana

Fungal infections interfere with the biosynthetic pathways in plants in various ways. Some fungi lower the production of insect-attracting volatiles (Dötterl et al., 2009), whereas others produce new, insect-behaviour modifying volatiles (Tasin et al., 2011). For example, Puccinia arrhenatheri (Uredinales: Pucciniaceae) infecting Berberis vulgaris (Berberidaceae) and P. monoica infecting Boechera stricta (Brassicaceae) trigger production of behaviour-modifying volatile chemicals, such as 2-phenylethanol and phenylacetaldehyde (Roy & Raguso, 1997; Raguso & Roy, 1998). Epiphyas postvittana adults show varying responses to various plant volatiles (Suckling et al., 1996; Jordan et al., 2009). Acetic acid and benzaldehyde produced by the plant as part of their normal metabolism attract Lepidoptera. For instance, benzaldehyde plays a role in attracting Chrysodeixis includens (= Pseudoplusia includens), Helicoverpa armigera, and Trichoplusia ni (Lepidoptera: Noctuidae),
and *Grapholista molesta* (= *Cydia molesta*) (Lepidoptera: Tortricidae) (Haynes *et al*., 1991; Burguiere *et al*., 2001; Meagher, 2002; Piñero & Dorn, 2007) and acetic acid plays a role in attracting *Cydia pomonella* (Lepidoptera: Tortricidae) to different varieties of *Malus domestica* (Rosaceae) (Knight *et al*., 2011). Gravid females of *E. postvittana* are deterred from *B. cinerea*-infected leaves of *V. vinifera* at a significant level and the deterrence level increases with the intensity of infection (Rizvi *et al*., 2015a). Although, *E. postvittana* is an established polyphage, which lays eggs indiscriminately on various plant species (Foster & Howard, 1999), volatile compounds such as hexanal, nonanal, and benzaldehyde influence olfactory and oviposition behaviour in it (Suckling *et al*., 1996). Hexanal and nonanal exposed to *E. postvittana* adult females, under artificial conditions, encouraged oviposition, whereas citral discouraged (Suckling *et al*., 1996). Decline in the levels of hexanal, nonanal, benzaldehyde, acetic acid, and the new biosynthesis of 1-octen-3-ol and 3-octanone in *B. cinerea*-infected *V. vinifera* leaves, as shown in the present study, could therefore play a role in deterring gravid females of *E. postvittana*. Monophagous, specialist-insect taxa are known to rely on olfaction, whereas this sensory modality is of low value in polyphagous taxa, such as *E. postvittana* (Ramaswamy, 1988; Foster & Howard, 1999). The response of female *E. postvittana* towards plants is also governed by leaf texture, especially smooth surface being preferred over rough (Foster *et al*., 1997). In a two-choice experiment of my previous study, significantly fewer number of *E. postvittana* females chose to lay eggs on *B. cinerea*-infected leaves than uninfected leaves of *V. vinifera* (Rizvi *et al*., 2015a) and no eggs occurred on infected sites of *V. vinifera* leaves. These suggest that olfactory cues are important for orientation, but tactile cues may play a vibrant role in directing oviposition.

### 4.3. *Botrytis cinerea* infection of *V. vinifera* influences the survival rate and larval performance of *E. postvittana*

Plants interact with microbes in various ways. The interacting microbes may have either beneficial or detrimental effects on insect performance. The insect can either prefer to feed on or avoid fungus-infected tissue. *Spodoptera exigua* prefers to lay
eggs on *S. rolfsii*-infected leaves of *A. hypogaea* and the larvae fed on infected leaves showed better survival, higher pupal mass and shorter time to pupation than those who fed on uninfected leaves (Cardoza *et al*., 2003a). In contrast, larvae of *Epirrita autumnata* (Lepidoptera: Geometridae) fed on the *Melampsoridium betulinum* (Pucciniales: Pucciniaceae) infected leaves of *Betula pubescens* (Fagales: Betulaceae) experience detrimental effect on life-history parameters (Lappalainen *et al*., 1995). An infected plant could still be a food source for the Lepidoptera, since the associated fungus could improve insect nourishment either by breaking the complex materials into simpler forms or by decreasing defense-compound levels (Cardoza *et al*., 2003b; Hatcher, 1995). The larvae of *E. postvittana* showed a better life-history performance when fed on 10% *B. cinerea*-infected leaves of *V. vinifera* (Rizvi *et al*., 2015a). However, with higher-infection level, the host-tissue quality could degenerate (Tasin *et al*., 2012). Cardoza *et al*., (2003) used a sub-lethal infection of *S. rolfsii* while rearing larvae of *S. exigua* and concluded that high intensity of fungal population may have a reversal effect on the larvae. *Botrytis cinerea* is a necrotrophic fungus and can kill the host by degrading plant tissue (Fournier *et al*., 2013). The caveat here is that I used a maximum of 60% infection level to determine volatiles, since beyond 60% infection level, the leaf abscesses. However, the choice of adult female in avoiding *B. cinerea*-infected leaves of *V. vinifera* appears to benefit the larvae because the time difference between oviposition and eclosion increases the infection level and consequently deteriorates *V. vinifera* tissue. In such a context, the choice of gravid females in avoiding *B. cinerea*-infected leaves of *V. vinifera* impresses as an adaptive strategy.

5. **CONCLUSION**

Infection by *B. cinerea* alters the volatile profile in the leaves of *V. vinifera*, which in turn modifies the oviposition behaviour of *E. postvittana*. The mortality rate of larvae fed on *B. cinerea*-infected leaves of *V. vinifera* was 100%. Gravid *E. postvittana* recognize volatiles arising from uninfected and *B. cinerea*-infected leaves of *V. vinifera* and choose to oviposit at sites, which enable the best performance of their offspring.
6. REFERENCES


Chapter 6

*Epiphyas postvittana* – *Botrytis cinerea* – *Vitis vinifera* interaction: the role of *B. cinerea* on the development of *E. postvittana* in synthetic nutritional media*

*Published paper (details below) presented with minor modifications.*

This page is intentionally left blank
1. INTRODUCTION

Incidence of *Epiphyas postvittana* on multiple fruit crops (e.g., *V. vinifera, M. domestica* (Rosaceae), several species of *Citrus* (Rutaceae) and *Pyrus* (Rosaceae)), a few vegetables (e.g., *P. sativum* (Fabaceae)), and flower crops (e.g., species of *Dahlia* and *Gerbera* (Asteraceae)) results in considerable economic loss (Brockerhoff *et al.*, 2011). *Epiphyas postvittana* is an Australian native tortricid but has spread to and established in New Zealand, England, Ireland, the Netherlands, and Sweden, and in Hawaii and California of USA (Danthanarayana, 1975; Suckling & Brockerhoff, 2010; Rizvi *et al.*, 2015a).

The larvae of Lepidoptera, which usually are voracious feeders, are so physiologically adapted that they utilize plant materials, which differ markedly in their nutritive value, water content, and defence compounds including several enzymatic inhibitors. The general mechanism of utilization of proteins and carbohydrates by the larvae of the Lepidoptera has been clarified (Jongsma *et al.*, 1995; Markwick *et al.*, 1998). When *E. postvittana* larvae were fed on a synthetic diet incorporated with proteinase and α-amylase inhibitors, the growth rates of the larvae was seldom affected, and the mortality rate was not significantly reduced. The larvae ‘compensated’ the lack of activity of proteinase and α-amylase through either hyper-production of inhibited enzymes or production of an inhibitor-insensitive form of an absent enzyme (Markwick *et al.*, 1998).

Lipid requirements of adult Lepidoptera are largely met during their larval stages (Ojeda–Avila *et al.*, 2003). Insects in general and Lepidoptera in particular lack the capacity to synthesize essential sterols to build key hormones and other lipid molecules that regulate development. The plant-feeding insects, therefore, acquire sterols (or sterol precursors) from their host plants and/or from the microbial symbionts (Mondy & Corio-Costet, 2000; Behmer & Nes, 2003). Polyunsaturated fatty acids, such as linoleic, and linolenic acids are vital for ecdysis and their deficiency leads to failure of ecdysis and wing deformities (Dadd, 1985; Canavoso *et al.*, 2001).
One essential nutritional requirement of plant-feeding insects is sterols. Cholesterol, a primary sterol, maintains cell-membrane integrity, regulates permeability, and acts as a precursor for hormones (Behmer & Nes, 2003). A mutualistic relationship among *B. cinerea—L. botrana—V. vinifera* has been demonstrated, wherein *L. botrana* has been shown to gain from *B. cinerea* for acquiring sterols, which in turn enhances the capacity of *L. botrana* to metamorphose rapidly by gaining greater biomass, lower pupal mortality, and greater egg production (Mondy & Corio-Costet, 2000). Incidence of *B. cinerea* in the diet of *L. botrana* influences diapause termination and thus adult emergence (Mondy & Corio-Costet, 2004). In general, ergosterol predominantly occurs in fungi and the most insects symbiotic with fungi have the ability to acquire ergosterol from fungi and metabolize it to cholesterol (Svoboda et al., 1995; Behmer & Nes, 2003). When *M. sexta* were reared on artificial diet that included ergosterol, a significant quantity of 7-dehydrocholesterol was derived from ergosterol (Svoboda et al., 1995). These findings indicate that the fungus could be a source of sterols to insects, although, in essence, a majority of insects can feed on plant tissues only and still perform.

Microorganisms influence the nutritional value of host plants, establishing either a positive or a negative relationship, ultimately reflecting on the fitness of plant-feeding insects. The interaction between the larvae of *E. postvittana* and the *B. cinerea*, both occurring on *V. vinifera* has demonstrated a mutual relationship between *E. postvittana* and *B. cinerea* (Rizvi et al., 2015b) with shorter larval period, heavier pupal mass, and greater fecundity rate. In the three-way interacting system involving *H. bicuris, S. latifolia*, and *M. violaceum* variations in the quantity of volatile compounds produced in *S. latifolia* due to infection by *M. violaceum* influence the behavior of *H. bicuris* negatively (Dötterl et al., 2009). These indicate that the fungi play a critical role in the development of Lepidoptera. Every possibility prevails that the fungi supplement the sterol requirements of insects in such intimate associations.

Keeping above in view, in the present study involving three interacting genomes — *E. postvittana, V. vinifera*, and *B. cinerea* — I hypothesize that although *E. postvittana* relies on *V. vinifera* as its principal host, *B. cinerea* influences the
growth of larvae and performance of adults of *E. postvittana*. I reared the larvae of *E. postvittana* — from egg to adult — on (i) a standard synthetic diet (diet A), (ii) the standard synthetic diet amended with a known volume of freeze-dried mycelial material of *B. cinerea* (Diet B) and (iii) the standard synthetic diet amended with a known volume of freeze-dried leaf material of *V. vinifera* only (Diet C). I also determined the preference of the larvae of *E. postvittana* towards Diets A, B, and C. To verify this, I sought answers to the following questions: (1) Does *B. cinerea* mycelial material enhance the life-history performance of *E. postvittana*? (2) Do the larvae of *E. postvittana* prefer to feed on a diet that includes the *B. cinerea* mycelial material? (3) Do the leaf materials of *V. vinifera* enable the life-history performance of *E. postvittana*?

2. MATERIALS AND METHODS

2.1. Insect culture

Eggs of *E. postvittana* were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O). The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm³) following the method described in (see Chapter 1). The cultures were maintained at 21±1°C, 60–80% RH, and 16L: 8D regimen. All tests were performed under identical environmental conditions. For all bioassays in this study, only the neonate larvae (<4 h after emergence) were used.

2.2. Lyophilization of samples of the mycelia of *B. cinerea* and leaves of *V. vinifera*

For this study, *B. cinerea* was grown in potato dextrose broth (Thermo Fisher Scientific, Thebarton, Adelaide, South Australia) in 500 ml sterile flasks fitted onto a mechanical shaker (90 rpm) for 10 d, filtered, and freeze-dried (Heto DryWinner, CT/DW 60E, Allerød, Denmark).

Sixty plants of *V. vinifera* were raised in plastic pots as described in (Chapter 4) (17 cm tall, 10 cm diameter) containing commercial potting mix (Osmocote, Plus
Organics Vegetable and Herb Mix, Sydney, Australia) in an insect-proof glasshouse at 23°C, 60% RH, and 16L:8D. At the start of experiments, the raised plants were four months old with several fully expanded leaves. Mature, dark-green leaves (9–12 cm long) were excised. In field conditions, the larvae of *E. postvittana* only selectively feed on intervenal tissues avoiding the major and minor veins. Therefore, petioles and recognizable veins from the leaves were sloughed off and the remaining leaf tissues were freeze-dried.

### 2.3. Larval-diet preparation

The synthetic diet for the larvae of *E. postvittana* was developed as described in Chapter 2. For diet B, freeze-dried powder of the mycelium of *B. cinerea* and for the diet C freeze-dried powder of leaves of *V. vinifera* was mixed with diet cooled to 50–55°C. Each molten diet was poured into three plastic containers of 20x15x7 cm³ each and allowed to cool further in a laminar air flow for 2 h. The composition of the control diet (Diet A) and the *B. cinerea*-enriched diet (Diet B) and *V. vinifera*-enriched diet (Diet C) is indicated in Table 6.1.

### 2.4. Larval preference: Two-choice experiment

The preference of neonate larvae of *E. postvittana* towards Diets A and B was measured following Harris *et al.* (1999) with minor modifications. Five g of solidified artificial diets A and B were placed at each end of the horizontal glass tube (30 long, 3 cm diameter) under ‘still-air’ condition. At the mid-point of the horizontal glass tube, a 2-cm wide circular port enabled the introduction of the larvae. The distance of the each diet from the port (midpoint) was 10 cm. Ten neonate larvae were introduced into the tube through the port, which was immediately sealed with Parafilm (Figure 6.3) and incubated at 21±1°C, 60–70% RH, and 16L: 8D. After 48 h, the location of the larvae was observed. This experiment was repeated 10 times.
2.5. Life history performance of *E. postvittana*:

2.5.1. *Growth and development*

Three batches of 30 neonate larvae (*n*=90) were distributed in three plastic containers that included either Diet A or B or C. Use of three containers, each with 30 neonate larvae, was necessary to minimize competition among the introduced larvae. The larvae were monitored every day until pupation. Upon pupation, the insects were gently removed from the diet containers and the mass of each pupa was measured, 0.1 mg sensitivity). The measured pupal mass was used as an indicator of adult-body mass, because adult body mass could not be obtained without killing them. Each pupa was quarantined to a glass vial (4 cm tall, 2 cm diameter), which was closed with a one-holed stopper covered by an aluminium sieve, and stored in the incubator at 22°C until emergence. Adults were sexed on emergence.

The following parameters were recorded: total survival rate (= adult emergence rate %), total development time (from the neonate larva to pupation, from the pupa to adult), mass of pupae, and adult sex ratio.

2.5.2. *Adult performance*

Adults were paired by placing a 24 h-old female and a male in a corrugate-walled Dixie™ cup sealed with Parafilm™. A water-soaked cotton wick was suspended into the cup through the Parafilm seal (Figure 6.1). The adult males and females used in these trials were randomly selected from among the emerged population. The number of eggs laid was counted daily. The quarantined adults were retained in Dixie cups until death. Eggs were incubated at 22°C, 16L: 8D regimen for 15 d to measure the fertility rate of females. Adult performance was measured using the fecundity rate, possible delay in oviposition (in d), and fertility of females (% of hatched eggs). The number of days from the date of emergence of adults from the pupal stage to death was also recorded.
2.7. **F₂ generation**

Twenty larvae from five females of F1 generation reared on Diet A or Diet B were continue on the respective diets to determine whether any similar effects prevailed in the F₂ generation. Growth parameters and adult performance, as indicated in paragraphs above, were measured.

2.8. **Statistical analysis**

Prior to statistical analysis, the distribution of data from each experiment was checked. None of the data sets, except pupal masses and delaying time before oviposition, was found normally distributed. Therefore, use of nonparametric methods was considered appropriate (Day & Quinn, 1989). A Kruskall–Wallis ANOVA followed by a Steel–Dwass multi-comparison test was selected to discriminate significant difference among Diets A, B and C in larval and pupal duration, adult life span, number of laid eggs, and larval emergence. Pupal masses and delay in time before oviposition were analyzed applying one-way ANOVA. A contingency table ($\chi^2$ test) followed by a Ryan multi-comparison test was applied to screen significant differences in the sex ratio, percentage of pupation and percentage of adult emergence. Fertility proportions in Table 6.2 and 6.3 were predicted and analyzed using regression model using generalized linear model (binomial distribution). In F₂ generation, a contingency table ($\chi^2$ test) was used to analyze sex ratio, percentage of pupation, and percentage of adult emergence, whereas the Kruskal-Wallis nonparametric test was applied in assessing the other life-history traits (Table 6.3). In two-choice experiment of larval preference, the choice of larvae of either Diets B or A were analyzed applying $\chi^2$ test. Analyses were made using JMP® software (Version 10.0.0, SAS Institute Inc) and GenStat® (VSN International 2012) (Hertfordshire, U.K.). Graphs and tables were generated in MS Excel 2013.
3. RESULTS

3.1. Larval preference: Two-choice experiment

Larvae of *E. postvittana* (n=100) showed no significant preference for Diet B (55.1%) compared to Diet A (44.9%), $\chi^2=1.02, p=0.31$, Figure 6.2

3.2. Life-history performance of *E. postvittana*:

3.2.1. Growth and development

The percentage of the larvae that survives from hatching to pupae was significantly greater when fed on Diet B (82.6%, $\chi^2=4.04, p=0.04$, Figure 6.3) and Diet C (88.7%, $\chi^2=7.05, p=0.008$, Figure 6.4) from those fed on Diet A (66.6%, Figure 6.4). The total larval duration of the larvae reared on Diet B or Diet C was significantly shorter than in the Diet A (Table 6.2). The duration of male development decreased 2.7 d on Diet B ($p=0.006$) and to 3.3 d on Diet C ($p>0.001$). Similarly, female development duration decreased by 3.0 d on Diet B ($p>0.001$) and 2.8 d on diet C ($p=0.006$). Only Diet C significantly affected the female pupal mass. The increase in female pupal mass was 14.8% ($p=0.001$) (Table 6.2). The survival rate from hatching to adult was also significantly higher in larvae fed on Diet B (73.3%, $\chi^2=5.56, p=0.01$, Table 6.2) and Diet C (74.6%, $\chi^2=6.5, p=0.01$, Table 6.2) compare to those fed on Diet A (54.6%, Table 6.2).

3.2.2. Adult performance

No diet had any effect on the sex ratio of adults and the length of the period prior to egg-laying whereas Diet B and C significantly increased fecundity rate (Table 6.2). The addition of *B. cinerea* or *V. vinifera* to the Diet A positively affected on the fertility and significantly increased ($p=0.001$) the larval emergence compared with the base diet (Diet A), although the larval emergence was significantly greater ($p=0.02$) in Diet B than Diet C (Table 6.2).
Table 6.1 The composition of the control diet (Diet A) and the *B. cinerea*-enriched diet (Diet B) and *V. vinifera*-enriched diet (Diet C).

<table>
<thead>
<tr>
<th>Materials</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (ml)</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Agar (g)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Casein (g)</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Wesson salt mixture (g)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cellulose (g)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Vanderzant vitamin mixture (g)</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Linoleic acid (ml)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Cholesterol (g)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>4N KOH (ml)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Sorbic acid (g)</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Paraben (g)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>95% Ethanol (ml)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptomycin (g)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Freeze-dried powder of mycelium of <em>B. cinerea</em> (g)</td>
<td>—</td>
<td>7.8</td>
<td>—</td>
</tr>
<tr>
<td>Freeze-dried powder of leaves of <em>V. vinifera</em> (g)</td>
<td>—</td>
<td>—</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Figure 6.1 Custom-made glass device for the oviposition of *Epiphyas postvittana* (not to scale). (cc–corrugated cup, dw–dental wick, ♀–female moth, ♂–male moth, ps–parafilm seal).
3.3. **F₂ Generation**

Larvae reared on Diet B developed faster than larvae raised on Diet A. The percentage of larvae that survives from hatching to pupae and pupae to adult were significantly greater when fed on Diet B \((p=0.003)\) from those fed on Diet A. *Botrytis cinerea* did not affect pupal mass, sex ratio of adult, pupal duration and the length of pre-oviposition period (Table 6.3). *Botrytis cinerea* enriched diet significantly increased fecundity rates \((p=0.008)\) and larval emergence \((p=0.004)\) compared with Diet A (Table 6.3).

4. **DISCUSSION**

In the present study, *B. cinerea* mycelial material used as larval food significantly affected the development and reproductive life-history traits of *E. postvittana*. The larvae reared on Diet B (*B. cinerea*-mycelial material enriched diet) survived better, developed faster, laid more number of eggs manifesting significantly greater fertility rates on becoming adults. However, the sex ratio, pupal mass, and adult-life span were not significantly different from those reared on Diet A (control). Diet C (*V. vinifera*-leaf enriched diet) also influenced the development of the larvae: larval mortality was reduced and developmental time of the larvae was short, pupal mass was increased; moreover the fecundity rate was greater than the larvae fed on Diet A. The F₂ generation of the larvae reared on Diet B showed similar effects in the life-history performance of the larvae as was in F₁ generation. No significant preference of larvae of *E. postvittana* for Diet B occurred.

4.1. *Botrytis cinerea* mycelia enhance the life-history performance of *E. postvittana*.

The quality of material fed during larval stages generally influences the life-history parameters of the Lepidoptera, such as survival, development, and adult performance (Thiéry & Moreau, 2005; Tasin et al., 2011). The quality of larval diet
Figure 6.2 Response of larvae of *E. postvittana* in a ‘two-choice’ experiment towards Diet A and Diet B.

Figure 6.3 Survival rate of larvae of *E. postvittana* reared on control (Diet A), fungus-enriched diet (Diet B), and leaf enriched diet (Diet C).
Table 6.2 Effects of larval food on life-history traits of *E. postvittana*

<table>
<thead>
<tr>
<th>Life-history traits</th>
<th>Larval food</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet A</td>
<td>Diet B</td>
</tr>
<tr>
<td>Larval duration (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32.7±0.7A</td>
<td>30.0±0.48B</td>
</tr>
<tr>
<td>Female</td>
<td>33.9±0.6A</td>
<td>30.91±0.4B</td>
</tr>
<tr>
<td>Pupal duration (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10.1±0.17</td>
<td>9.3±0.40</td>
</tr>
<tr>
<td>Female</td>
<td>9.5±0.20AB</td>
<td>9.0±0.15B</td>
</tr>
<tr>
<td>Pupal mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28.5±0.80</td>
<td>28.4±1.04</td>
</tr>
<tr>
<td>Female</td>
<td>42.4±1.47A</td>
<td>44.0±1.20A</td>
</tr>
<tr>
<td>Adult life span (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12.0±0.77</td>
<td>13.5±0.80</td>
</tr>
<tr>
<td>Female</td>
<td>10.1±0.45</td>
<td>11.7±0.60</td>
</tr>
<tr>
<td>Female sex ratio (%)</td>
<td>56.6±1.6</td>
<td>53.8±4.4</td>
</tr>
<tr>
<td>Surviving from hatching to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adult (%)</td>
<td>53.3±7.4A</td>
<td>72±6.2B</td>
</tr>
<tr>
<td>Delay in egg laying (d)</td>
<td>2.1±0.08</td>
<td>1.9±0.09</td>
</tr>
<tr>
<td>Number of eggs/female</td>
<td>300±34.3A</td>
<td>498±49.4B</td>
</tr>
<tr>
<td>No. of larvae emerged per</td>
<td>235±30.1A</td>
<td>435±41.6B</td>
</tr>
<tr>
<td>female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertility ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(number of larvae emerged/</td>
<td>0.77±0.04A</td>
<td>0.88±0.02B</td>
</tr>
<tr>
<td>female÷ number of eggs /</td>
<td></td>
<td></td>
</tr>
<tr>
<td>female)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Kruskal–Wallis ANOVA.
<sup>b</sup> Steel–Dwass test for non-parametric multicomparsion.
<sup>c</sup> Ryan multicomparison test for proportion.
<sup>d</sup> Generalized linear model (binomial distribution)
Table 6.3 Effects of larval food on life-history traits of F2 generation of *E. postvittana*

<table>
<thead>
<tr>
<th>Life-history traits</th>
<th>Larval food</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet A</td>
<td>Diet B</td>
</tr>
<tr>
<td>Larval duration (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32.6±0.8</td>
<td>29.8±0.44</td>
</tr>
<tr>
<td>Female</td>
<td>33.8±0.5</td>
<td>31.0±0.6</td>
</tr>
<tr>
<td>Pupal duration (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10.1±0.15</td>
<td>9.3±0.37</td>
</tr>
<tr>
<td>Female</td>
<td>9.2±0.15</td>
<td>9.0±0.15</td>
</tr>
<tr>
<td>Pupal mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28.1±0.82</td>
<td>29.7±0.92</td>
</tr>
<tr>
<td>Female</td>
<td>41.6±1.3</td>
<td>43.3±1.15</td>
</tr>
<tr>
<td>Adult life span (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11.3±0.63</td>
<td>14.0±0.62</td>
</tr>
<tr>
<td>Female</td>
<td>10.3±0.45</td>
<td>11.8±0.59</td>
</tr>
<tr>
<td>Female sex ratio (%)</td>
<td>53.6±2.1</td>
<td>52.5±2.9</td>
</tr>
<tr>
<td>Surviving from hatching to pupa (%)</td>
<td>69±5.2</td>
<td>78±8.7</td>
</tr>
<tr>
<td>Surviving from hatching to adult (%)</td>
<td>61.8±6.2</td>
<td>76.2±5.2</td>
</tr>
<tr>
<td>Number of eggs/female</td>
<td>314±26.5</td>
<td>494±50.11</td>
</tr>
<tr>
<td>No. of larvae emerged per female</td>
<td>243±26.4</td>
<td>433±48.5</td>
</tr>
<tr>
<td>Fertility ratio (number of larvae emerged/female÷ number of eggs/female)</td>
<td>0.78±0.04</td>
<td>0.86±0.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>Kruskal–Wallis ANOVA.
<sup>b</sup>χ² test.
<sup>c</sup>Steel–Dwass test for non-parametric multicomparison.
<sup>d</sup>Ryan multicomparison test for proportion.
<sup>e</sup>Generalized linear model (binomial distribution)
determines the body size and fecundity of adult stages of most insects (Awmack & Leather, 2002) and the Lepidoptera are no exception (Tasin et al., 2011). For instance, oocyte production in *Euphydryas editha* and *Speyeria mormonia* (both Lepidoptera: Nymphalidae) depends on the quality of food consumed during larval period (Boggs, 1997). In my earlier study, I have shown that *B. cinerea*-infected berries of *V. vinifera* influence the feeding behaviour of the larvae of *E. postvittana* favouring their development and survival (Rizvi et al., 2015b).

Sterols are essential for insects, since they are the critical precursors of hormones necessary for metamorphosis and reproduction, but insects lack the ability to produce them. Plant-feeding insects meet their sterol needs, more commonly that of cholesterol, using sterols from either their host plants or the fungi with which they are associated symbiotically (Corio-Costet et al., 1987; Behmer & Nes, 2003). In the present study, Diet B influenced life-history parameters such as decrease in the total duration of development, mortality rate and increase in fecundity and larval emergence rates in *E. postvittana*.

Insects live feeding on fungi without any specialized relationship or they may feed on a specific fungus and their relationship ranges from deriving simple materials to complex molecules such as complex lipids. Different species of fungi act as a source of sterols (Morales-Ramos et al., 2000; Mondy et al., 1998a) particularly ergosterol, a common sterol used by various insects (Behmer & Nes, 2003). Six different sterols have been characterized from *B. cinerea* and the major sterol is ergosterol (Mondy & Corio-Costet, 2000). Most of the insects symbiotic with fungi have the ability to metabolize the ergosterol obtained from fungi into cholesterol (Behmer & Nes, 2003; Svoboda et al., 1995).

Deficiency of cholesterol accelerates mortality and decelerates ecdysis (Canavoso et al., 2001). The neonate larvae of *Heliothis zea* (Lepidoptera: Noctuidae) do not moult to the next stage when sterols are absent in the diet and cease growth when older larvae are raised on a cholesterol-free diet (Ritter & Nes, 1981). Therefore, Ritter and Nes (1981) concluded that the larval survival and number of moults, which a larva may complete in a given time span, are dependent on sterol concentration in the diet. Ritter and Nes (1981) reinforced that at least
0.01% of cholesterol is the minimal dietary requirement for the larvae to grow and pupate. Cholesterol concentration up to 0.4% did not adversely affect the survival and growth rates of *H. zea* larvae. In the present study, I used 0.06% cholesterol ensuring that a non-limiting quantity of the sterol was available in the experimental diet. Decrease in mortality rate and larval-developmental time indicates that Diet B supplies, in high likelihood, sterols necessary for larval survival and growth. Moreover the Diets A, B, and C, used in the present study, replace maize oil with linoleic acid. Maize oil is known to supply cholesterol to developing Lepidoptera (Ritter & Nes, 1981).

Diet B influenced multiple changes, such as a decrease in the larval-developmental time and mortality. However, no significant effect was evident in pupal mass, whereas a significant increase in fecundity and fertility rates occurred in *E. postvittana* larvae, when reared on Diet B. Generally, insect fecundity rate depends on adult-female sizes and rate of increment in body size (Honêk, 1993; Karlsson, 1987). In *E. postvittana* raised on Diet B, increase in fecundity did not match with adult-female sizes. A similar result has been shown in the larval population of *L. botrana* reared on purified sterols from *B. cinerea*; no significant difference occurred in pupal mass but the fecundity rate increased significantly (Mondy & Corio-Costet, 2000). Many species of Scolytinae (Coleoptera: Curculionidae) are dependent on ergosterol produced by their fungal symbionts for successful oocyte development, oviposition, larval development, and pupation (Kok, 1979). Cholesterol is the principal sterol stored in many insect eggs (Behmer *et al*., 1999; Jouni *et al*., 2002), indicating that cholesterol is vital for embryogenesis. The greater rate of larval emergence when raised on Diet B in the present study also reinforces the vitality of cholesterol in eggs, which could be serving as a precursor to ecdysteroids regulating embryogenesis (Sall *et al*., 1983). It is not clear why Diet B induced shorter developmental period of larvae than Diet A. This is possible due to one of the following reasons: (1) greater levels of sterols in Diet B acting as phagostimulants (Mondy *et al*., 1998b); (2) greater levels of bio-available cholesterol.
4.2. The larvae of *E. postvittana* show no preference to feed on the diet containing the mycelial material of *B. cinerea* (Diet B)

Diet B did not attract any significant number of larvae in the two-choice experiment. In polyphagous insects, the quality and quantity of deterrent compounds in plants determine the host-plant range (Bernays & Chapman, 1994). *Epiphyas postvittana* is a polyphagous tortricid and its larvae disperse randomly on hatching (Suckling & Brockerhoff, 2010). Visual cues influence the orientation of larval movement (Harris *et al*., 1995). Mobile larvae of *E. postvittana* did not distinguish between potential host and non-host from a distance (Harris *et al*., 1995), whereas random movement of *E. postvittana* larvae led them to unfavourable sites used in experiment (Suckling & Ioriatti, 1996; Harris *et al*., 1997). That *E. postvittana* larvae did not show an initial preference for control diet over the diet enriched with *Bacillus thuringiensis* δ-endotoxins (cryIAc and cryIBa) and over time the larvae rejected δ-endotoxin-including diets and gathered on control diet (Harris *et al*., 1997) suggesting that olfactory cues along with gustatory cues influence their decision enabling them to either progress with the feeding or not (Suckling & Ioriatti, 1996; Harris *et al*., 1999). In my two-choice experiment, the diet possibly lacked any deterrent or anti-nutritional compound that could have influenced the feeding preference of the larvae of *E. postvittana*; and this seems a more likely explanation of my results of larval preference.

4.3. The leaves of *V. vinifera* enhance the life-history performance of *E. postvittana*

*Epiphyas postvittana* can complete its life cycle, feeding solely on *V. vinifera* leaves and berries (Rizvi *et al*., 2015b; Rizvi & Raman, 2015); however, when the larvae were fed exclusively on *B. cinerea*-infected berries of *V. vinifera*, they manifested positive effects on key life-history traits, such as greater survival rate, pupal weight, and fecundity (Rizvi *et al*., 2015b). The larvae of *S. exigua*, when fed on *Sclerotium rolfsii*-infected leaves of *A. hypogaea* manifested similar positive effects in life-history performance with greater survival rate and pupal mass, and lower developmental time than those fed on uninfected leaves of *A. hypogaea* (Cardoza *et al*., 2015b).
In my study, the larvae fed on Diet C survived better, attained heavier pupal mass in females with a shorter developmental period than those fed on Diet A. Diet C also increased the oviposition rate compared with Diet A matching with the results of Thiéry and Moreau (2005) in which young berries of V. vinifera (Cabernet–Sauvignon) increased female pupal mass and fecundity rate, and reduced developmental period in L. botrana. Although Diet B influenced key life-history parameters, the present data do not support that the fungus involvement is critical for the survival and growth of E. postvittana.

5. **CONCLUSION**

In Australian vineyards, E. postvittana adults frequently encounter B. cinerea during oviposition, and E. postvittana larvae co-occur with B. cinerea feeding on their hyphae and spores (Bailey et al., 1996; Rizvi et al., 2015a, b), establishing a three-way interacting system. Although, E. postvittana can complete its life cycle by feeding solely on the leaves and berries of V. vinifera, for a better life-history performance, they can feed on the spores and hyphae of B. cinerea possibly to meet the sterol needs of developing E. postvittana.

6. **REFERENCES**


Chapter 7

Oviposition preference and larval performance of *Epiphyas postvittana* on *Botrytis cinerea* infected berries of *Vitis vinifera*

*Published paper (details below) presented with minor modifications.

This page is intentionally left blank
1. INTRODUCTION

The link between host-plant preference and offspring performance is central in insect–plant interaction studies (Thompson, 1988). The ‘decision’ made on the suitability of the host plant by a plant-feeding insect is critical for its progeny (Gripenberg et al., 2010). Among various plant-feeding insects, pre-adult mobile stages have limited opportunities to explore newer food sites in case the oviposited sites were inappropriate. The gravid female, therefore, usually, selects a site that will service the nutritional needs of her offspring (Mayhew, 1997). The preference—performance hypothesis (also referred to as the ‘mother-knows-the-best’ hypothesis) proposes that oviposition preference should correspond with host suitability for offspring development; the adult females, thus, maximize the fitness of their offspring by ovipositing at the most appropriate host sites (Jaenike, 1978). Energy stored during pre-adult mobile (‘larval’ hereafter) stages is utilized during non-feeding pupal stages. The quality of food consumed during larval stage also contributes to reproductive performance. Reproduction in several plant-feeding taxa has been demonstrated to be closely linked to feeding preferences during their larval stages (Awmack & Leather, 2002). For instance, oocyte production depends on the quality of food consumed during larval periods; bulk of nitrogen and carbon components of eggs is derived from the nutrients obtained during larval period (Boggs, 1997). Many mated females usually prefer either one or related few among diverse plants for oviposition. Nevertheless, that oviposition preference relates to offspring performance is currently being debated (Mayhew, 1997). For instance, a gravid female’s decision in the choice of a plant could be based on reasons favouring her performance than that of her offspring (Valladares & Lawton, 1991; Mayhew, 1997). This selfish-motherhood hypothesis has been reinforced by Scheirs et al. (2004), and Scheirs et al. (2004). In such alternate scenarios, insects fail to make the seemingly optimal choice and they oviposit on a plant, which will achieve the optimal growth and development of their offspring.

Among plant-feeding insects, chemical cues from plants are vital for host selection. Female insects use different sensory cues to locate and select the ‘most suitable’ plant and the site for nourishment and/or oviposition (several examples cited in Schoonhoven et al., 2005; Bruce et al., 2010; also see Chapter One).
Olfaction, contact chemoreception, and vision play decisive roles in this process. From a distance, in the specific instance of *Epiphyas postvittana* (Lepidoptera: Tortricidae) (Foster *et al*., 1997) and *Lobesia botrana* (Lepidoptera: Tortricidae) (Tasin *et al*., 2011), volatiles from the host enable insects to locate their hosts for oviposition, but when at the correct host surface, contact cues influence their decision enabling them to either progress with the oviposition or not (Rojas *et al*., 2003; Rizvi *et al*., 2015). Volatiles of host plants are influenced and modified by various factors, such as infection by microbes, which can alter host-plant quality. In the natural environment and agricultural contexts, plants often encounter several organisms. Among these, insects and fungi concurrently attack plants, resulting in a three-way interaction among plants, plant-feeding arthropod, and plant-infecting microbe (See Chapter 1). The fungi, in such interacting systems, can induce multiple variations in plant chemistry (Cardoza *et al*., 2003b; Raman *et al*., 2012). Such variation in host quality alters volatile composition, which is recognized by plant-feeding insects by their olfactory capacity, which, in turn, can change the preference behaviour in insects (Najar-Rodriguez *et al*., 2010; Qawasmeh *et al*., 2012). The fungus-induced plant volatiles can act as either attractants or deterrents for plant-feeding insects (Tack & Dicke, 2013; Qawasmeh *et al*., 2014). For example, *A. hypogaea* when infected by *S. rolfsii* releases specific terpenes, which act as attractants to *S. exigua* (Cardoza *et al*., 2002). *Botrytis cinerea*, degrades the host-plant cell-wall hydrocarbons and releases 3-methyl-1-butanol, which arises in plant tissues consequent to decay (Magan & Evans, 2000; Tasin *et al*., 2012). 3-methyl-1-butanol released by *V. vinifera* when infected by *B. cinerea* deters oviposition by *L. botrana* (Tasin *et al*., 2012). On the other hand, fungal mycelia can be a source of nourishment for the lepidopteran larvae, enabling them to synthesize sterols and vitamins (Svoboda *et al*., 1994).

In Australian vineyards, the active adults of *E. postvittana* encounter *B. cinerea* during oviposition, whereas their larvae co-occur with *B. cinerea* feeding on their hyphae and spores (Bailey *et al*., 1996; Rizvi *et al*., 2015; Chapter 1). In Australia, *E. postvittana* often coincide with berry production and nearly 65% of *E. postvittana* larvae move to berries of *V. vinifera* inflicting intense productivity losses (Buchanan, 1977; Bailey *et al*., 1996).
Botrytis cinerea induces grey-mould disease on V. vinifera affecting leaves and fruits (Fournier et al., 2013). Botrytis cinerea–V. vinifera association eventuates in the production of various volatiles, which have been shown to influence the oviposition behaviour of E. postvittana (Rizvi et al., 2015) and L. botrana (Tasin et al., 2012). A mutualistic relationship between L. botrana and B. cinerea has been shown: the larvae of L. botrana benefit from B. cinerea because their mycelia supply sterols such as ergosterol enabling the metamorphosis of L. botrana, whereas L. botrana helps B. cinerea dispersing its spores (Mondy & Corio-Cost et al., 2000). Moreover, chewing of V. vinifera tissues by L. botrana larvae inflicts wounds facilitating the establishment of the fungus (Fermaud & Le Menn, 1992).

Therefore, I propose that the gravid individuals of E. postvittana recognize the volatiles from uninfected (control) and B. cinerea-infected berries of V. vinifera and choose to oviposit at the most appropriate site enabling the best performance of their offspring. This was tested by evaluating the relationship between oviposition preference and larval performance of E. postvittana on uninfected berries and infected berries of V. vinifera. In keeping with the above, I sought answers to the following: (1) Do the females of E. postvittana prefer to oviposit on berries of V. vinifera infected by B. cinerea? (2) Do the larvae of E. postvittana prefer to feed on berries of V. vinifera infected by B. cinerea? (3) Do the B. cinerea-infected berries affect the survival and life-history performance of E. postvittana larvae?

2. MATERIALS AND METHODS

2.1. Insect culture

Eggs of E. postvittana were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O). The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm\(^3\)) following the method described in(see details in Chapter 1). The cultures were maintained at 21±1°C, 60–80% RH, and 16L: 8D regimen. All tests were performed under identical environmental conditions.
Adult males and females were not exposed to either uninfected or *B. cinerea*-infected berries of *V. vinifera*, prior to experimentation.

2.2. **Preparation of conidial suspension**

The conidial suspension was prepared in sterile water following the method described in Chapter 3 and adjusted to $10^6$ conidia/ml.

2.3. **Infection of *V. vinifera* berries by *B. cinerea***

Berries of *V. vinifera* of phenological stage 85 (Lorenz *et al.*, 1994) were collected from CSU—O Chardonnay vineyard. Berries of *V. vinifera* were surface sterilized with 1% sodium hypochlorite (NaClO) solution for 5 min and washed with sterile water (3x). Using a sterile scalpel, four scars were scratched on the skins of each berry and were sprayed immediately with the prepared conidial suspension of *B. cinerea* in the horizontal-laminar airflow system. Uninfected berries were sprayed with sterile water. The infected and the uninfected berries were placed in a zip-lock plastic bag (35x40 cm) and incubated at 22°C and 12L:12D. After 9−11 d, the berries that manifested infection symptoms were used in different bioassays. Following Mondy *et al.*, (1998) and Tasin *et al.*, (2012), I used berries with visually evident grey-mould sporulation in the behavioural bioassays. The berry clusters with varying numbers of berries were used in the experiments, but were standardized by using clusters of nearly the same mass (90±8 g).

2.4. **Oviposition behaviour**

2.4.1. **Two-choice experiment**

The experiments were conducted in glass devices assembled suiting the requirements, using Pyrex glassware, at CSU—O research laboratory at 21±1°C, 60–70% RH, and 16L:8D. The assembled glass device consisted of two wide-mouth glass jars (10 diameter, 18 cm high) connected to each other by a glass tube (3 cm
diameter, 30 cm long). Each jar was lined with aluminium mesh screen (Figure 7.1). The mouths of the jars were also covered with the same aluminium mesh screen and were further wrapped with Parafilm, enabled with a hole to receive one end of the glass tube. The aluminium screen was necessary to distract females from ovipositing on the wall of the jar. This precaution was imperative because smooth-textured surfaces, similar to glass, stimulate oviposition by *E. postvittana* (Foster *et al.*, 1997). Aluminium mesh has been used in similar studies (Rojas *et al.*, 2003) testing the oviposition behaviour of *S. frugiperda*, Mondy *et al.* (1998) used latticed cages while testing the oviposition behaviour of *L. botrana*. I used the same material in both jars that included uninfected and *B. cinerea*-infected berries to minimize the effect of mesh in each treatment. Parafilm, an established odourless thermoplastic filmy material (Yoo & Nam, 2012), was used to prevent the interference of any external volatiles. Clusters of uninfected and infected berries were placed at the bottom of the two wide-mouth jars. At the mid-point of the glass tube, a circular (2.0 cm diameter) port was cut for introducing gravid *E. postvittana* enabling them to choose either infected or uninfected berries. Five live adult females were gathered in a clean glass test tube (2.0 diameter, 15 cm long). The diameter of this test tube was the same as that of the port in the horizontal glass tube. The port was set to face downwards. The test tube including the adult moths was held tight to the port. By virtue of their vertical-climbing behaviour, the adult moths crawled one after another into the horizontal tube, each taking about 2 min to achieve this task. The movement of moths from the vertical tube into the horizontal tube was individualistic and they did not influence the other in any obvious manner. In other words, c. 2 min was adequate for each moth to crawl one after the other from the vertical tube into the horizontal tube. By the time, the next one followed into the horizontal tube, the earlier crawled moth had adequate time to make its choice of moving towards one of the ‘baits’ (infected and uninfected berries) located at either end of the horizontal tube. The port was sealed using Parafilm. After 72 h, the introduced females were removed carefully from the jars. Choices made by the females were recorded by counting the numbers of moths in each jar; the numbers of eggs laid on the infected and uninfected berries were counted under a stereo-binocular microscope. This experiment was repeated eight times using freshly assembled wide-mouth glass jars.
and the horizontal glass tube. In each replication, the infected and uninfected berries used as baits were randomly allocated to the two jars.

2.4.2. *No-choice experiment – rate of oviposition*

A no-choice experiment was done to determine the rate of oviposition on infected and uninfected berries of *V. vinifera*, using glass jars (1000 ml). Clusters of infected and uninfected berries of similar mass were hung in two identical jars from the aluminium mesh used to cover the mouth of each jar, followed by a Parafilm wrapping. A gravid *E. postvittana* was introduced into the beaker and the mouth of jar was covered (Figure 7.2). Each treatment was repeated eight times. After 72 h, the moths introduced into the jars were removed and the numbers of eggs laid on the berries were counted, as described in the preceding paragraph.

2.4.3. *No-choice experiment – effect of volatiles on oviposition*

The rate of oviposition of *E. postvittana* in response to the volatiles emitted from the infected and uninfected berries was assayed following the method described in Chapter 3.

2.4.4. *Olfactory response of adults of E. postvittana*

The olfactory response of adult males of *E. postvittana* (24–48 h old) to *B. cinerea*-infected and uninfected berries of *V. vinifera* were measured using a Y-tube olfactometer. Two clusters of volatile sources, viz., *B. cinerea*-infected and uninfected berries of *V. vinifera* of similar mass were placed, one in each of the source containers. Compressed air (flow rate 400 ml/min) was pumped into the source containers via the tube inlets (Figure 7.3). Male moths were introduced individually through the downwind end of the Y-tube and their behaviour was observed for 15 min. Moths that remained at the end of the stem of the Y-tube for 30 min were deemed unresponsive and excluded from the tests. Females of *E.
postvittana have been shown to be less responsive and active than the males (Danthanarayana, 1976; Gu & Danthanarayana, 1990). In the pilot trials made using the Y-tube (n=10), adult females showed no response in the nominated 15 min time frame, and therefore they were excluded from Y-tube olfactometer trial.

2.5. Larval behaviour

2.5.1. Two-choice experiment

The preference of neonate larvae (<2 h) of E. postvittana towards B. cinerea-infected and uninfected berries was tested following the method described in Chapter 3. This experiment was repeated 10 times.

Larval behaviour towards B. cinerea-infected and uninfected berries of V. vinifera was further verified through another experiment. A Petri dish (90 mm0020diameter) was lined with damp-filter paper. One B. cinerea-infected and one uninfected berry of V. vinifera were placed at the two opposite points, 5 mm away from the wall of Petri dish (Figure 7.4). A neonate E. postvittana larva was placed at the centre of the Petri dish, the lid placed on top, and sealed with Parafilm. After 24 h, the Petri dish checked for the position of the larva noted. This experiment was repeated 100 times.

2.5.2. Transmission of conidia of B. cinerea

Transmission of conidia from infected to uninfected berries of V. vinifera was evaluated. Thirty individuals of 4th-instar larvae of E. postvittana that were raised on the semi-synthetic diet (see section ‘Insect culture’) ensuring that they were 100% clean, totally devoid of B. cinerea were fed on uninfected or B. cinerea-infected berries of V. vinifera for 48 h. Each larva was then transferred using a sterile camel-hair brush onto a surface-sterilized berry in a Petri dish, under the sterile conditions of the horizontal-laminar airflow. The larvae were enabled to feed for 48 h on either B. cinerea-infected or uninfected berries. The larvae were removed from the berries and the berries were incubated individually in sterile Petri
dishes sealed with Parafilm and incubated at 21°C for 7 d. The number of berries with visible levels of \textit{B. cinerea}-infection was recorded. Visible levels of infection on berries were determined under the stereo-binocular microscope using sporulation of \textit{B. cinerea} on the berries as a trait (Khazaeli \textit{et al.}, 2010).

2.6. \textbf{Larval development}

This experiment was done to determine the effect of \textit{B. cinerea}-infected berries on the life-history performance of \textit{E. postvittana}. \textit{Botrytis cinerea}-infected (9–11 d) and uninfected berries were placed on separate Petri dishes. One <2-h old larva was released on each Petri dish and allowed to develop until pupation at 21±1°C, 60–70% RH, and 16L:8D. Every 7 d, the dehydrating berries in the Petri dishes were changed and frass removed. Survival of the larvae was also checked. One hundred larvae were used in each treatment. The larvae were allowed to pupate. The dates of pupation, number of pupae, and pupal mass were recorded. Because precise measurements of the mass of active adults were difficult to obtain, the mass of the pupa was used as an indicator of adult-body mass. Each pupa was isolated to a stopper glass vial (2 cm diameter, 4 cm long) until emergence. Adult emergence and adult-sex ratio in percentage were recorded. The adults were sexed and paired in a corrugated-walled Dixie cup enabling mating and oviposition. The adults that were enabled to oviposit were subsequently measured for their performance: fecundity rate, possible delays in oviposition (measured in days), fertility (measured as the number of emerged larvae; the eggs were incubated for 15 d at 21±1°C, 60–70% RH, and 16L:8D enabling larval emergence) and percentage of ovipositing females. Number of days from the date of emergence of adults from the pupal stage to death was also recorded.

2.7. \textbf{Statistical analysis}

In the oviposition bioassay, the numbers of eggs laid on \textit{B. cinerea}-infected and uninfected berries in the two-choice experiment were analysed using paired
sample ‘t’ test. Independent sample ‘t’ test was applied to measure the significant difference in the number of eggs laid on *B. cinerea*-infected and uninfected berries in the no-choice experiment and the experiments measuring the effect of volatiles. Data from the Y-tube olfactometer, choice of the female moths for *B. cinerea*-infected or control berries in oviposition bioassay and choice of larvae for *B. cinerea*-infected or control berries in larval bioassay were analyzed applying $\chi^2$ test. Transmission of conidia from *B. cinerea*-infected berries to uninfected berries was analysed by contingency table ($\chi^2$ test). Non-linear regression was applied to discriminate the significant difference in the mortality rate of larvae between *B. cinerea*-infected and uninfected berries of *V. vinifera* using the equation ‘$y=a+bx$’, where $x$ is the time (d), $y$ is mortality and A, B and R (rate of curvature) are estimated parameters. A contingency table ($\chi^2$ test) was used to analyse female sex ratio, percentage of pupation, percentage of adult emergence and percentage of Egg-laying females whereas independent sample t-test was used in all other life-history traits (Tables 6.1, 6.2 and 6.3). Analyses were conducted with SPSS statistic 17.0 (2007) and GenStat (VSN International, 2012) (Hertfordshire, U.K.). Graphs were generated in MS Excel 2013.

3. RESULTS

3.1. Oviposition behaviour

3.1.1. Two-choice experiment

Gravid *E. postvittana* significantly chose uninfected berries (70%) compared with *B. cinerea*-infected berries (30%, $n=40$, $\chi^2=6.40$, $p=0.011$). Gravid *E. postvittana* laid significantly more number of eggs on uninfected berries of *V. vinifera* than on *B. cinerea*-infected berries (uninfected vs infected: 114 vs 18, d.f.=7, $t=9.13$, $p<0.001$, Figure 7.5a).

3.1.2. No-choice experiment – rate of oviposition

Gravid *E. postvittana* laid significantly more number of eggs on uninfected berries compared with *B. cinerea*-infected berries of *V. vinifera* (uninfected vs infected, 58 vs 9, d.f.=14, $t=8.51$, $p<0.001$, Figure 7.5b).
3.1.3. **No-choice experiment − effect of volatiles on oviposition**

Gravid *E. postvittana* laid significantly more number of eggs on the wall of the Dixie cup in the ambience of volatiles emitted from uninfected berries, compared with *B. cinerea*-infected berries of *V. vinifera* (uninfected vs infected, 347 vs 220, d.f.=18, t=3.03, \( p=0.007 \), Figure 7.5c).

3.1.4. **Olfactory response of adults of Epiphyas postvittana**

Adult-male moths responded positively and significantly to the volatiles from uninfected (control) berries compared with *B. cinerea*-infected berries (uninfected vs infected, 70% vs 30%, \( n=40 \), \( \chi^2=6.4 \), \( p=0.01 \)). Female moths (\( n=10 \)) remained in the Y–tube stem and did not respond to volatiles from uninfected or *B. cinerea*-infected berries.

3.2. **Larval behaviour**

3.2.1. **Two-choice experiment**

In glass tube experiment, the neonate larvae of *E. postvittana* (\( n=100 \)) moved towards uninfected and *B. cinerea*-infected berries (uninfected vs infected, 43% vs 57%, \( \chi^2=1.96 \), \( p=0.161 \)).

In Petri dish experiment, the neonate larvae of *E. postvittana* (\( n=100 \)) moved towards uninfected (control) berries and *B. cinerea*-infected berries (control vs uninfected, 55% vs 45%, \( \chi^2=1 \), \( p=0.317 \)).

3.2.2. **Transmission of conidia of Botrytis cinerea**

The uninfected berries, when fed upon by the larvae which were previously fed on infected berries, manifested the grey-mould disease (86.6%). On the contrary, those uninfected berries when fed upon by the larvae which were previously fed on
Figure 7.1 Custom-made glass device for the two-choice experiment of adults of *Epiphyas postvittana* (not to scale). am—aluminium mesh, gj—glass jar, hgt—horizontal-glass tube, ib—infected berries, m—moth, pe—port of entry, ps—Parafilm seal, ub—uninfected berries.

Figure 7.2 Custom-made glass device used in the ‘no-choice experiment — rate of oviposition of *Epiphyas postvittana*’ (not to scale). am—aluminium mesh, b—beaker, ib—infected berries, m—moth, ps—Parafilm seal, ub—uninfected berries.

Figure 7.3 Y—tube experiment to determine olfactory response of adult *E. postvittana* (not to scale). ap—air pump, ib—infected berries, ub—uninfected berries, m—moth, tc—Teflon plug, st—source tube, tt—Teflon tube, yt—Y tube.
Figure 7.4 Petri dish test for the larval preference towards uninfected and *B. cinerea*-infected berries of *V. vinifera* (not to scale). dfp—damp filter paper, ib—infected berries, l—larvae, pd—Petri dish, ub—uninfected berries.

Figure 7.5 Mean number of eggs laid by *E. postvittana* in [a] two-choice experiment, [b] no-choice experiment — rate of oviposition, [c] no-choice experiment — effect of volatiles on oviposition in response to uninfected (■) and *B. cinerea*-infected (▲) berries of *V. vinifera*. Error bars denote SEM. **p<0.01, ***p<0.001.
uninfected berries manifested the grey-mould disease at a low percentage (6.6%, \( n=30, \chi^2=16.1, p<0.001 \)); even this low percentage of disease manifestation could have been possibly due to contamination.

### 3.3. Larval development

The larvae reared on *B. cinerea*-infected berries developed quicker (male \( p<0.001 \); female \( p<0.001 \)) than the larvae raised on uninfected berries of *V. vinifera* (Table 7.1). The mortality rate of larvae fed on uninfected berries (\( y=48.91-49.18*0.89^x \)) was significantly greater (\( p<0.001 \)) than those reared on *B. cinerea*-infected berries of *V. vinifera* (\( y=29.63-29.45*0.85^x \), where \( x=\text{day} \) and \( y=\text{mortality rate} \) (Figure 7.6). Pupal mass of males and females of *E. postvittana* raised on *B. cinerea*-infected berries of *V. vinifera* were significantly greater (male \( p<0.0001 \); female \( p<0.001 \)) than those of the larvae that were reared on uninfected berries (Table 7.2). Rate of pupation and adult emergence of larvae raised on *B. cinerea*-infected berries were significantly higher (pupation \( p=0.005 \); adult emergence \( p=0.003 \)) compared with those reared on uninfected berries. *Botrytis cinerea* infection did not affect the sex ratio of adults, percentage of the gravid females, and the length of pre-oviposition period (Tables 7.2 and 7.3). *Botrytis cinerea*-infected diet significantly increased the fecundity rates (\( p=0.01 \)) and larval emergence (\( p=0.02 \)) compared with control diet (Table 7.3).

### 4. DISCUSSION

Gravid *E. postvittana* prefer to oviposit on uninfected berries relative to *B. cinerea*-infected berries of *V. vinifera*. The larvae of *E. postvittana* show no significant preference for uninfected berries; they transmit the conidia of *B. cinerea* to berries in the vicinity. The insects survive better, develop faster, attain a heavier pupal mass, and lay more numbers of eggs when reared on *B. cinerea*-infected berries than those reared on uninfected berries of *V. vinifera*. 
Figure 7.6 Mortality rate of larvae reared on *B. cinerea*-infected (■) or uninfected (□) berries of *V. vinifera*.

Table 7.1 Larval developmental time, pupal duration and adult life span (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected berries of *V. vinifera*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval developmental time (days)</th>
<th>Pupal duration (days)</th>
<th>Adult life span (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Uninfected berries</td>
<td>33.4±0.6</td>
<td>41.2±0.9</td>
<td>10.2±0.2</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected berries</td>
<td>27.9±0.5</td>
<td>33.4±0.8</td>
<td>9.7±0.1</td>
</tr>
<tr>
<td>Statistic</td>
<td>t=6.6</td>
<td>t=5.9</td>
<td>t=1.7</td>
</tr>
<tr>
<td><em>p</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Table 7.2 Pupal mass, percentage of larvae surviving from hatching to pupae, percentage of larvae surviving from hatching to adult and female sex ratio (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected berries of *V. vinifera*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pupal mass (mg)</th>
<th>% surviving from hatching to pupa</th>
<th>% surviving from hatching to adult</th>
<th>Female sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected berries</td>
<td>17.5±0.5</td>
<td>23.1±0.7</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected berries</td>
<td>21.4±0.8</td>
<td>30.4±1.6</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>Statistic</td>
<td><em>t</em>=3.9</td>
<td><em>t</em>=6.0</td>
<td>$\chi^2$=7.81</td>
<td>$\chi^2$ = 8.9</td>
</tr>
<tr>
<td><em>p</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Uninfected berries of *V. vinifera*

$t = $ Independent sample t-test

$\chi^2 = $ Contingency table ($\chi^2$)

Table 7.3 Adult performance (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected berries of *V. vinifera*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Egg-laying females (%)</th>
<th>Delay in egg laying (days)</th>
<th>Fecundity (no. eggs per female)</th>
<th>No. of larvae emerged per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected berries</td>
<td>88</td>
<td>2.2±0.1</td>
<td>246.1±25.7</td>
<td>183.2±27.9</td>
</tr>
<tr>
<td><em>B. cinerea</em> Infected berries</td>
<td>94</td>
<td>2.3±0.2</td>
<td>326.8±19.5</td>
<td>255.6±18.1</td>
</tr>
<tr>
<td>Statistic</td>
<td>$\chi^2$=0.3</td>
<td><em>t</em>=0.175</td>
<td><em>t</em>=2.5</td>
<td><em>t</em>=2.3</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.58</td>
<td>0.86</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$t = $ Independent sample t-test

$\chi^2 = $ Contingency table ($\chi^2$)
4.1. Females of *E. postvittana* are deterred from ovipositing on *B. cinerea*-infected berries of *V. vinifera*

Several factors affect host selection in generalist plant-feeding insects for feeding and oviposition. Among such insects, the preference for certain hosts, the preference to lay eggs on plants, and the numbers of eggs laid at specific locations are essentially driven by an assessment of cues from the host using visual, olfactory, and tactile stimuli. *Botrytis cinerea*-infected berries of *V. vinifera* evoked avoidance behaviour in the gravid *E. postvittana* deterring oviposition. Fungi induce changes in the volatile profiles in plant systems, which in turn alter the behaviour of the Lepidoptera that interact with a fungus—plant system (Mondy *et al.*, 1998; Cardoza *et al.*, 2003b). In the three-way interacting system involving *Hadena bicruris*, *S. latifolia*, and *M. violaceum* variations in the quantity of volatile compounds produced in *S. latifolia* have been shown to alter the behaviour of *H. bicruris* (Dötterl *et al.*, 2009). On the other hand, in the three-way interacting system involving *L. botrana*, *V. vinifera*, and *B. cinerea*, variations in the quality of volatile compounds produced have been shown to alter the behaviour of *L. botrana*, in addition to variations in quantities of volatiles produced (Tasin *et al.*, 2011). In the *E. postvittana—V. vinifera—B. cinerea* system, *B. cinerea* is capable of killing *V. vinifera* in conducive environments (Fournier *et al.*, 2013). During infection, *B. cinerea* degrade the carbohydrates such as pectin and cellulose and liberate hydrocarbons (Magya & Bene, 2006), which, in turn, transform and release behaviour-modifying compounds such as 3–methyl–1–butanol and ethanol (Carlile *et al.*, 2001; Jansen *et al.*, 2009; Tasin *et al.*, 2012). For example, gravid females of *L. botrana* were deterred from ovipositing on *B. cinerea*-infected berries (Tasin *et al.*, 2012). Such physiological changes and the consequent release of volatiles form *B. cinerea*-infected berries may responsible for the deterrence behaviour in gravid *E. postvittana*. Odours from the berries infected with *B. cinerea* also deterred the males, which could influence the mating behaviour, since the males use plant volatiles to distinguish environments where the likelihood of finding the females is higher (Ansebo *et al.*, 2004).

Tactile stimuli play a key role in the choice of host for egg laying by plant-feeding insects (Rojas *et al.*, 2003). That oviposition by *E. postvittana* is influenced
by tactile cues has been shown by Foster and Howard (1999). Surface texture plays a role in the oviposition behaviour of gravid *E. postvittana*, which prefers to lay eggs on smooth surfaces of varicose texture, rather than on rough and hairy surfaces such as the abaxial sides of the leaves of *V. vinifera* (Rizvi *et al.*, 2015). This corroborates with the pattern of oviposition in *E. postvittana* noted in natural environments on the leaves of *V. vinifera* (Danthanarayana, 1975) and *M. domestica* (Tomkins *et al.*, 1991). The fungal mycelia growing on the surfaces of either leaves or berries of *V. vinifera* mimic hairiness, and possibly that acts as a physical barrier in the preference of *E. postvittana* during oviposition (Rizvi *et al.*, 2015). This explains why so few eggs of *E. postvittana* occur on the berries of *V. vinifera* infected by *B. cinerea* as compared with the numbers of eggs laid on the corrugated walls of Dixie cups in the ambience of volatiles from *B. cinerea*-infected berries.

4.2. The larvae of *E. postvittana* show no significant preference to *B. cinerea*-infected berries of *V. vinifera*

*Botrytis cinerea*-infected berries influence the behaviour of *E. postvittana* developmental stages variously. The larvae of *E. postvittana*, unlike the adults, show no significant attraction to uninfected berries. These results also match with my earlier findings (Rizvi *et al.*, 2015), wherein I reported discordance in oviposition behaviour versus larval-feeding behaviour in *E. postvittana*, when tested on uninfected and *B. cinerea*-infected leaves of *V. vinifera*. The larvae of *E. postvittana* do not necessarily follow the choice made by their mother and move to other sites on *V. vinifera* (Harris *et al.*, 1997; Foster & Howard, 1999). Here, I cannot disregard the suggestion made by Scheirs and De Bruyn (2002) that oviposition occurs consequent to attraction of food source to adults. Adult plant-feeding insects often prefer to feed and oviposit on such sites, which favour their long-term fitness and in consequence, make poor choices for their progeny.
4.3. *Botrytis cinerea*-infected berries of *V. vinifera* affect the survival and life-history performance of *E. postvittana* larvae

I aimed at verifying the preference—performance hypothesis (Jaenike, 1978) that plant-feeding insects such as *E. postvittana* have evolved to choose the right host for oviposition that will enable the best performance of the offspring. According to Jaenike, the oviposition-preference should match with host suitability for offspring development. Among polyphagous plant-feeding insects, mated females often exhibit a preference for oviposition among different host plants, the oviposition preference usually synchronizes with the best performance of offspring, although evidences negating Jaenike (1978) also exist (Larsson & Ekbom, 1995; Leyva et al., 2000; Mayhew, 1997; Gripenberg et al., 2010). The choice of larvae for food may substantially differ from the adults particularly when the larvae and adults feed on different parts of a plant (Mayhew, 1997), for example, in *E. postvittana*. A poor relationship between preference of adults for oviposition and preference of larvae for feeding by *E. postvittana* using *V. vinifera* and several other plants without the involvement of *B. cinerea* has been shown (Foster & Howard, 1999). Gravid females of *E. postvittana* oviposited indiscriminately on host and non-host plants; in contrast, the larvae of *E. postvittana* showed a greater level of preference to *V. vinifera* than to other plants tested. The present work testing the preference behaviour of *E. postvittana* in the presence of *B. cinerea* on *V. vinifera* during oviposition reinforces Foster and Howard’s (1999) findings that gravid adults of *E. postvittana* do not follow the preference—performance hypothesis. Among plant-feeding insects, optimal foraging and optimal oviposition are not independent; but optimal foraging influences host selection more than optimal oviposition in effect (Scheirs et al., 2004). The selfish-motherhood hypothesis, was tested by Scheirs et al. (2000) who suggested that variation in the adult preference is highly correlated with adult performance rather than with offspring performance in host plant selection. Whether the poor relationship between oviposition preference and the offspring performance is due to this selfish-motherhood behaviour of adult *E. postvittana* remain unknown. It thus appears that additional studies are needed to elucidate the role of food sources on both the long-term fitness of egg-laying females and the oviposition response in the presence of these resources.
According to Yang et al. (2013) avoidance of fungus-infected tissue by gravid insects during oviposition could be an adaptive strategy for the fitness of the offspring. Biere and Tack (2013) also suggest a similar outcome that the level of fungal infection can influence the oviposition preference of insects. In a specific context, Tasin (2012) showed that L. botrana displayed significant level of deterrence of the 7–9 d old B. cinerea-infected V. vinifera berries, whereas no significant inhibition occurred when 1–3 d old infected berries were supplied to the gravid adults of L. botrana. On the other hand, the fungus associated with the plant could also facilitate the nutrition of the insect, either by breaking complex materials into simpler forms or by decreasing the level of defence compounds in the plant (Cardoza et al., 2003a). In the present study, since I have used 9–11 d old B. cinerea-infected berries of V. vinifera, it would be appropriate to suggest that the difference between the time of oviposition and that of egg hatch potentially enhances the level of infection of V. vinifera berries by B. cinerea. Therefore, the larval food quality could have potentially deteriorated with accelerated level of infection (Tasin, 2012). This possibly explains why gravid adults avoid infected berries for oviposition.

My results point to a mutualistic relationship between the larvae of E. postvittana and B. cinerea. Although, adults of E. postvittana do not favour this relationship and do not prefer to oviposit on B. cinerea-infected berries, the selection behaviour of V. vinifera by the larvae differs from that of adults, either because they have different host requirements or because they feed on different plant organs. The larvae of E. postvittana vector the conidia trapped among hairs on their body surfaces, in gut and faeces, and spread the conidia among susceptible berries (Rizvi et al., 2015; Bailey et al., 1997). In the present study, I show that feeding on B. cinerea-infected berries of V. vinifera induce multiple physiological changes in the E. postvittana, such as an increase in fecundity, the emergence rate of larvae, a decrease in the total duration of development and mortality. Larval diet plays an important role in determining the size and fecundity of most insects (Svoboda et al., 1994) and the Lepidoptera in particular (Mondy et al., 1998), because the energy required for oviposition and egg development is mainly derived from reserves accumulated during their larval stages. Under such a circumstance, the fungi
possibly act as a source of nutrition and food supplement to the larvae of *E. postvittana*. Mutualistic relationship between an insect and a fungus is common among different insects (Svoboda *et al.*, 1994; Mondy & Corio-Costet, 2000). Inability of insects to synthesize sterols constrains them to depend on exogenous sources such as plants and/or their symbionts. Working on the larvae of *L. botrana* Mondy *et al.* (1998) found that *L. botrana* larvae are mutualistically related to *B. cinerea* on *V. vinifera* for sterol source from the fungus. Based on this study, I propose that the relationship of *E. postvittana* with *B. cinerea* infected *V. vinifera* is driven by mutualism.

In my earlier study (Rizvi *et al.*, 2015b), I indicated that the larvae of *E. postvittana* showed a better life-history performance when fed on 10% *B. cinerea*-infected leaves of *V. vinifera* (Rizvi *et al.*, 2015a) but the larvae could not survived when they fed on 70% *B. cinerea*-infected leaves of *V. vinifera* (Chapter 5). I also showed that the synthetic diet incorporated with freeze-dried mycelium of *B. cinerea* improved larval development and increased fecundity in adults of *E. postvittana* (Rizvi & Raman, 2015a). In response to *B. cinerea* infection, *V. vinifera* leaves accumulate many defence compounds including several possible anti-insectan compounds, such as chitinase and β-1, 3-glucanase (Busam *et al.*, 1997; Derckel *et al.*, 1999; Vihervuori *et al.*, 2013), whereas with the onset of ripening (véraison) of berries, some of the defence compounds do not manifest; or possibly they get modified into non-defensive compounds (Jeandet *et al.*, 1995). The accumulation of these compounds in the leaves in response to the *B. cinerea* infection could be one reason for the mortality of larvae of *E. postvittana* while rearing them on *B. cinerea*-infected leaves of *V. vinifera*.

5. **CONCLUSION**

In this study, I show that the gravid females of *E. postvittana* avoid and do not prefer to oviposit on *B. cinerea*-infected berries while the rate of oviposition is significantly decreased in the ambiance of volatiles from *B. cinerea*-infected berries. The larvae of *E. postvittana* showed no preference for uninfected berries. Larvae vector the conidia of *B. cinerea* and infect the healthy berries. Furthermore, the
larvae which fed on *B. cinerea*-infected berries survived better, developed faster, attained a heavier pupal mass and laid more eggs than those reared on uninfected berries of *V. vinifera*. Gravid *E. postvittana* recognize the volatiles arising from uninfected and *B. cinerea*-infected berries of *V. vinifera* but choose to oviposit at sites which need not lead to the best performance of their offspring.

6. REFERENCES


This page is intentionally left blank
Chapter 8

Volatile from *Botrytis cinerea* infected and uninfected berries of *Vitis vinifera* influencing the oviposition behaviour of *Epiphyas postvittana*.

*Published paper (details below) presented with minor modifications.
This page is intentionally left blank
1. INTRODUCTION

Insects use sensory cues, such as olfactory, contact chemoreceptory, and visual, to ‘identify’ and ‘assess’ food, mates, predators, competitors, and sites for oviposition (Schoonhoven et al., 2005). Among plant-feeding insects, the preference for certain plants, decision to lay eggs, and the number of eggs laid on a substrate are based on host-cue assessment (Renwick & Chew, 1994; Schoonhoven et al., 2005). Compared with vision and contact chemoreception, olfaction largely influences the assessment of host quality based on volatile organic compounds (Beyaert et al., 2010). Based on sensory cues of the insect, the choice of a suitable plant is key to the success of performance of its progeny (Gripenberg et al., 2010). Odour comprises several volatile compounds in specific ratios (Knudsen et al., 1993). Variation in host quality can affect the composition of these volatiles (Dötterl et al., 2009). Insects by their olfactory capacity can perceive such variations (Tasin et al., 2011).

In the natural environment, insects and fungi are associated with a majority of plants, establishing a three-way interacting system (See more description in Chapter 1). In an earlier experiment, I demonstrated that the synthetic diet incorporated with B. cinerea improved the fitness of larvae of Epiphyas postvittana (Lepidoptera: Tortricidae) (Rizvi & Raman, 2016a).

Fungal infection induces changes in plant metabolism (Dötterl et al., 2009; Raman et al., 2012) recognize by plant-feeding insects, which, in turn, alter their behaviour (Najar-Rodriguez et al., 2010). Volatiles, arising in plant–fungi interactions, act as either attractants (Mondy et al., 1998; Cardoza et al., 2003a) or deterrents (Dötterl et al., 2009; Rizvi et al., 2015a) for insects, particularly in three-way interacting systems. Infection of V. vinifera by B. cinerea induces the production of behaviour-modifying volatiles that influence the host choice made by the Tortricidae (Tasin et al., 2012; Rizvi et al., 2015a, b). I have shown that gravid adults of E. postvittana generally do not lay eggs on B. cinerea infected V. vinifera (Rizvi et al., 2015a, b; Rizvi & Raman, 2016b). A similar result was shown in L. botrana–B. cinerea–V. vinifera interacting system (Tasin et al., 2011). In the same paper, Tasin et al. (2011) indicated that females of L. botrana were attracted to
yeast-infected *V. vinifera*. These findings suggest that the presence of volatiles from a specific microbial community may be a critical cue for eliciting oviposition.

Plants infected by fungi emit volatile compounds, such as alcohols, carbonyls, and hydrocarbons (Morath *et al*., 2012). Among the alcohols, ethanol and 3-methyl-1-butanol are the most common volatiles released during fungal infection of plants, including that by *B. cinerea* (Tasin *et al*., 2012). Behavioural alteration induced by such volatiles (ethanol and 3-methyl-1-butanol) is known in several insects (El-Sayed *et al*., 2005).

In the present study, I used a three-way interacting system of *E. postvittana—V. vinifera—B. cinerea* to determine the effect of *B. cinerea* infection on the volatiles produced in the berries of *V. vinifera* and how that infection could affect the olfactory response of *E. postvittana*. In vineyards, *E. postvittana* adults and *B. cinerea* often occur within the same time frame when oviposition takes place.

*Epiphyas postvittana*, light brown apple moth, is a generalist herbivore (plant-feeder), native to Australia (Suckling & Brockerhoff, 2010). *Epiphyas postvittana* is predominantly a nocturnal flier with a flight activity pattern dependent on the time of the day. The peak flight-activity of *E. postvittana* was recorded at 20.00 h (Australian Eastern Standard Time) for the winter (June—August) generation and at 21.00-24.00 h for the spring (September—November) and summer (December—February) generations in Victoria, Australia (Danthanarayana, 1976). *Epiphyas postvittana* can recognize volatile compounds produced by plants. At least three odour receptors have been identified from the antennae of *E. postvittana* that recognize volatiles, particularly methyl salicylate (Jordan *et al*., 2009), a common plant-stress signal that arises in response to abiotic and biotic factors (Shulaev *et al*., 1997). *Botrytis cinerea* occurs worldwide and infects more than 200 crops (Chapter 1; Fournier *et al*., 2013). *Botrytis cinerea* induces grey-mould on *V. vinifera* (Fournier *et al*., 2013), which in Australian vineyards costs $50 million/annum in management (Scholefield & Morison, 2010).

Keeping the above in view, I hypothesized that adults of *E. postvittana* differentially respond to volatiles produced by infected and uninfected berries of *V. vinifera*, and this response is reflected in host site(s) influencing oviposition. To test
this, I sought answers to the following questions. First, does infection of *B. cinerea* modify the volatile chemistry of *V. vinifera*? Second, do volatiles of *B. cinerea* infected berries inhibit attraction and oviposition in *E. postvittana*? To secure answers, I determined the volatiles of *B. cinerea* infected and uninfected berries of *V. vinifera*; verified the olfactory behaviour of *E. postvittana* in a wind tunnel and the oviposition behaviour towards *B. cinerea* infected and uninfected berries of *V. vinifera*; and tested the role of ethanol and 3-methyl-1-butanol, using laboratory standards, on the oviposition behaviour of *E. postvittana* via a two-choice experiment.

2. MATERIAL AND METHODS

2.1. Insect rearing

Eggs of *E. postvittana* were obtained from laboratory cultures maintained at Charles Sturt University, Orange Campus (CSU–O). The neonate larvae were maintained on a semi-synthetic diet in plastic containers (35x20x4 cm³) following the method described in Chapter 2. The cultures were maintained at 21±1°C, 60‒80% RH, and 16L: 8D regimen. All tests were performed under identical environmental conditions.

Adult males and females were not exposed to either *B. cinerea* infected or uninfected berries of *V. vinifera* before experimentation. The room where the assays were conducted was maintained at 21±1°C, 60‒80% RH, and 16L: 8D regimen. In any assay, photoperiod was not reversed.

2.2. Fungus culture and preparation of conidial suspension

The conidial suspension was prepared in sterile water following the method described in Chapter 3 and adjusted to 10⁶ conidia/ml.
2.3. Infection of V. vinifera berries with B. cinerea

Berries of V. vinifera (90±15 g) of phenological stage 85 (Lorenz et al., 1994) were inoculated with B. cinerea spores and incubated at incubated at 22° C and 12L:12D using the method describes in Chapter 7. After 1—3, 9–11 d of incubation, the berries that manifested disease symptoms were used in different assays.

2.4. Headspace collection and volatile profiling

Volatiles emanating from infected and uninfected berries were collected using solid-phase microextraction (SPME) technique as described earlier in Chapter 5. Briefly, four uninfected or B. cinerea infected berries were confined to 20 ml stoppered and sterilized, glass vials for 60 min. The SPME fibre was preconditioned at 250° C for 5 min in the injector port of the GC. Volatiles from the berries were collected at 30° C for 30 min by placing the preconditioned SPME fibre in the headspace of either uninfected or infected berries. After collection, the fibre was immediately inserted into the injector port of the GC–MS interfaced to a Gersal MPS 2XL autosampler) at 250° C and the volatiles were assayed using the same method as described earlier in Chapter 5. Six replicates each of uninfected and B. cinerea infected berries were analyzed for volatiles over a four day period. A blank was run after each analysis ensuring that no contaminant adhered to the SPME fibre. Volatiles were identified at >80% probability using NIST library. Compounds to be tested in behavioural assays were selected according to their relative concentration in the headspace and their behavioural activity reported (Epsky et al., 1998; Nout & Bartelt, 1998; Tasin et al., 2011, 2012).

2.4.1 Identification of the compounds

A majority of compounds was identified according to their mass spectra and retention times by comparing with authentic standards from Sigma-Aldrich (St. Louis, Missouri) and Merck KGaA (Darmstadt, Germany). Those compounds whose authentic standards were unaffordable were identified consulting NIST library
(>80% probability) and AnalyzerPro databases. Further confirmation of these compounds, which were not verified using authentic standards, was made by calculating Kováts retention index (C₅ — C₂₅) on BP21 column and relating them with the retention indices listed in NIST library. The source (or the method) used to determine the identity of specific compounds is notified in results, Table 8.1.

2.5. **Wind-tunnel assay**

Behaviour towards *B. cinerea* infected (mild or intense) and uninfected berries of *V. vinifera* were verified in a custom-made wind tunnel (see detail described in Chapter 5). Air at a velocity of 25 cm/s was propelled at the upwind end of the wind tunnel. Two bunches of berries as odour stimuli were suspended at 20 cm distance from the upwind end, separated from each other by 30 cm in the width axis of the tunnel. Two hours before the end of the photophase, either adult males or gravid females of *E. postvittana* were placed in the downwind end of the tunnel in a 250 ml glass beaker covered by a pierced Parafilm sheet enabling aeration, to acclimate them to the tunnel.

At scotophase the Parafilm sheet was removed enabling *E. postvittana* to fly freely in the tunnel for 30 min. *Epiphyas postvittana* behaviour was scored for (1) no activity (confined to the beaker), (2) activity (*E. postvittana* fly upwind <20 cm), (3) activity (*E. postvittana* fly upwind 20–60 cm), (4) activity (*E. postvittana* fly upwind 60–120 cm), and (5) activity (*E. postvittana* fly upwind 120–180 cm; 170 cm earmarked the odour source, viz., the suspended berries). Four adult males or gravid females of *E. postvittana* were tested per run and 10 runs (n=40) were tested against either *B. cinerea* infected (either mild or intense) or uninfected berries of *V. vinifera*. When each test run was set, the beaker including adults of *E. postvittana* was placed at different points along the width of the tunnel (80 cm).
2.6. Oviposition behaviour

2.6.1 Two-choice experiment

Two-choice experiments were conducted to determine the preference of *E. postvittana* towards *B. cinerea* infected and uninfected berries of *V. vinifera* following the method described in Chapter 7.

2.6.2. Laboratory standard volatiles in the presence of uninfected *Vitis vinifera* berries

To determine the oviposition behaviour of *E. postvittana* towards 3-methyl-1-butanol and ethanol in the presence of uninfected berries of *V. vinifera*, I conducted an oviposition choice assay using a screw-neck max recovery vials (2 mL) containing 3-methyl-1-butanol (>99%) (10 or 100 µg) or ethanol (>99.8%) (10 or 100 or 1000 µg) (Sigma-Aldrich, St. Louis, Missouri) in paraffin oil (Tasin *et al*., 2012; Abraham *et al*., 2014). A 2 mL glass vial with paraffin oil served as control. One bunch of uninfected berries was placed with a glass vial containing either 3-methyl-1-butanol+paraffin oil or ethanol+paraffin oil or paraffin oil in the jars (Figure 8.1). Oviposition assays were conducted as described under ‘Two-choice experiment’ in Chapter 7. Four gravid females of *E. postvittana* were used in each experiment. This experiment was repeated eight times using freshly assembled, clean jars, and horizontal tubes. In each replicate, positions of the laboratory standard volatile and blank control were randomly allocated.

2.6.3. Laboratory standard volatiles in the absence of uninfected *Vitis vinifera* berries

To determine the oviposition behaviour of *E. postvittana* towards 3-methyl-1-butanol and ethanol, I conducted an oviposition-behaviour experiment without *V. vinifera* berries. Two screw-neck max recovery vials (2 mL) with 3-methyl-1-butanol (10 or 100 µg)+paraffin oil or ethanol (10 or 100 or 1000 µg)+paraffin oil or paraffin oil (control) were placed in a corrugated-walled Dixie cup. The cups were
covered with the aluminium mesh screens and Parafilm (Figure 8.2). The oviposition behaviour was assessed as described under ‘Two-choice experiment’ in Chapter 7. Four gravid females of *E. postvittana* were used in each experiment. This experiment was repeated eight times using adults from the same generation over four days.

### 2.7. Statistical Analysis

Prior to statistical analysis, the data from each experiment were checked for normality. Initial normality tests were made applying Shapiro-Wilk’s test. On getting skewed results, paired Wilcoxon rank sum non-parametric tests were applied to discriminate differences in numbers of eggs laid during oviposition assays. A contingency table ($\chi^2$ test) followed by a Ryan multi-comparison test was applied to screen for significant differences in each behavioural step of adults of *E. postvittana* in the wind-tunnel experiment.

### 3. RESULTS

#### 3.1. Volatile profile

I detected 16 compounds from the uninfected and 24 compounds from *B. cinerea* infected berries of *V. vinifera*. The volatile profile of *B. cinerea* infected berries varied greatly both qualitatively and quantitatively. Ethanol, 3-methyl-1-butanol, 2-phenylethanol, and ethyl acetate were dominant in *B. cinerea* infected berries. 1-hexanol, 3-Hexen-1-ol (*E*), and 2-ethyl-1-hexanol were dominant in uninfected berries. Laboratory standards of ethanol and 3-methyl-1-butanol were used as olfactory stimuli in the oviposition assay (Table 8.1). All of the compounds emitted by the *B. cinerea* infected berries were detected in at least one other infected sample.
3.2. Wind tunnel assay

The differences between flights of gravid females of *E. postvittana* towards uninfected and mildly infected berries were not significantly different ($\chi^2=5.98$, $p=0.20$). On the other hand, a significant inhibition in attraction was measured in female of *E. postvittana* towards intensely infected berries compared with uninfected berries of *V. vinifera* ($\chi^2=9.82$, $p=0.04$, Figure 8.3). The flight pattern of *E. postvittana* males was not significantly different when tested for uninfected or *B. cinerea* infected berries (mild, $\chi^2=2.59$, $p=0.62$; intense, $\chi^2=3.53$, $p=0.47$).

3.3. Oviposition behaviour

3.3.1. Two-choice experiment

When visual, olfactory, and tactile sensory cues were available, the duration of infection significantly influenced the behaviour of females of *E. postvittana*. No significant difference in preference occurred when mildly infected berries (55%) were offered against uninfected berries of *V. vinifera* (45%, $n=40$, $\chi^2=0.05$, $p=0.82$). In contrast, gravid *E. postvittana* were attracted significantly greater to uninfected berries (70%) compared with intensely infected berries (30%, $n=40$, $\chi^2=6.40$, $p=0.011$, Figure 8.4).

Mildly infected berries did not significantly deter oviposition of *E. postvittana* (control vs infected, 68 vs 52, $t=0.83$, $p=0.47$, Figure 8.5), but the intensely infected berries significantly deterred oviposition (uninfected vs infected, 85 vs 14, $t=33.6$, $p<0.001$, Figure 8.5).

3.3.2. Laboratory standard volatiles in the presence of Vitis vinifera berries

I found significant effects of 3-methyl-1-butanol and ethanol in the presence of *V. vinifera* volatile on the oviposition behaviour of *E. postvittana*. The females of *E. postvittana* exhibited a significant preference for the odour of paraffin oil+uninfected berries (control; 68.7%) compared with 1000 µg ethanol+paraffin oil (31.3%, $\chi^2=4.5$, $p=0.03$, Figure 8.6), whereas, no significant differences were
Figure 8.1 Custom-made glass device used in the two-choice experiment with adults of *Epiphyas postvittana* (not to scale). am—aluminium mesh, c—control, gj—glass jar, hb—uninfected berries, hgt—horizontal-glass tube, m—moth, pe—port of entry, ps—Parafilm seal, sv, synthetic volatile.

Figure 8.2 Custom-made glass device used in the two-choice experiment with adults of *E. postvittana* (not to scale). am—aluminium mesh, c—control, gj—glass jar, hb—uninfected berries, hgt—horizontal-glass tube, m—moth, pe—port of entry, ps—Parafilm® seal, sv, synthetic volatile.

Figure 8.3 Olfactory response of females of *Epiphyas postvittana* towards *Botrytis cinerea* infected berries (9–11 d old infection) and uninfected berries of *V. vinifera* in the wind tunnel. Within each behavioural step, circle labelled with different letters are significantly different ($P < 0.05$).
observed in the preference for paraffin oil+uninfected berries (control; 56.2%) compared with 100 µg ethanol+paraffin oil (43.8%, \( \chi^2=0.5, p=0.47 \), Figure 8.6) and paraffin oil+uninfected berries (control; 48%) compared with 10 µg ethanol+paraffin oil (52%, \( \chi^2=0.9, p=0.34 \), Figure 8.6). Similarly, there were no significant differences between controls and a 10 µg dose of 3-methyl-1-butanol (control vs dose10 µg, 56.3% vs 43.7%, \( \chi^2=0.5, p=0.47 \), Figure 8.7). In contrast, a 100 µg dose of 3-methyl-1-butanol+paraffin oil significantly inhibited the attraction (control vs dose100 µg, 71.8% vs 28.2%, \( \chi^2=6.4, p=0.01 \), Figure 8.7).

In the ambience of visual, olfactory, and tactile sensory cues, only the 1000 µg dose of ethanol+paraffin oil affected the oviposition of *E. postvittana* (dose 10 µg vs control, 82 vs 68, t=0.83, p=0.47; dose 100 µg vs control, 92 vs 82, t=0.06, p=0.81; dose 1000 µg vs control, 17 vs 130, t=25.08, p<0.001, Figure 8.8). Only 100 µg of 3-methyl-1-butanol+paraffin oil deterred oviposition (dose10 µg vs control, 63 vs 83, t=0.43, p=0.52; dose 100 µg vs control, 26 vs 76, t=6.4, p=0.02, Figure 8.9).

### 3.3.3. Laboratory standard volatiles in the absence of berries of *V. vinifera*

Laboratory standards of ethanol and 3-methyl-1-butanol were used as olfactory stimuli in the oviposition assay. In the absence of *V. vinifera* berry volatiles, ethanol did not affect the preference of females of *E. postvittana*. In contrast, I found a significant attraction towards a 10 µg dose of 3-methyl-1-butanol+paraffin oil (72%) compared with the paraffin oil (control, 28%, \( \chi^2=6.12, p=0.01 \), Figure 8.10). No significant attraction occurred towards the 100 µg dose of 3-methyl-1-butanol+paraffin oil (56%) compared with the paraffin oil (control, 44%, \( \chi^2=0.5, p=0.47 \), Figure 8.10).

No significant differences were found in number of eggs laid on the control versus any dose of ethanol+paraffin oil (10 or 100 or 1000 µg). Females of *E. postvittana* laid significantly more eggs only in the ambience of 10 µg of 3-methyl-1-butanol+paraffin oil, compared with the paraffin oil (control, (dose10 µg vs control, 156 vs 93, t=8.2, p=0.01; dose100 µg vs control, 174 vs 143, t=0.63, p=0.45, Figure 8.11).
4. DISCUSSION

This study pertains to host-preference behaviour of *E. postvittana* in relation to *B. cinerea* infected and uninfected berries of *V. vinifera*. To gain an understanding of the host-seeking behaviour, I characterized the organic volatile compounds in the uninfected and infected berries of *V. vinifera*. I also characterized the role of olfactory cues used by *E. postvittana*.

4.1. *Botrytis cinerea* infection changes the volatile profile of *V. vinifera*

Volatile released during plant–fungus interaction transmit messages of plant quality to the feeding and/or ovipositing insects. It is known that some fungal induced variations in plants can modify plant volatiles (Cardoza et al., 2003a; Raman et al., 2012). For instance, infection of *S. latifolia* by *M. violaceum* suppresses the production of principal insect-attracting volatiles in *S. latifolia*, such as lilac aldehyde and lilac alcohol (Dötterl et al., 2009). Whereas, fungi such as *P. arrhenatheri* and *P. monoica* trigger the production of behaviour-modifying volatile chemicals such as 2-phenylethanol and phenylacetaldehyde upon infecting *B. vulgaris* and *B. stricta*, respectively (Roy & Raguso, 1997; Raguso & Roy, 1998). Tasin et al. (2012) reported that a known behaviour-modifying volatile, 3-methyl-1-butanol, produced by infected *V. vinifera* berries influenced oviposition behaviour of *L. botrana*.

In the present study, the infection of *V. vinifera* by *B. cinerea* generated several volatile compounds. Alcohols constituted >80% of volatiles in *B. cinerea* infected berries, among which ethanol predominated at 72.2% followed by 3-methyl-1-butanol at 8.6%. This finding concurs with the high levels of ethanol (c. 80%) reported for *F. culmorum* -infected *T. aestivum* (Börjesson et al., 1989). Similar examples referring to the predominance of ethanol in fungus-infected plants
Table 8.1 Volatile compounds detected from *Botrytis cinerea* and uninfected berries of *V. vinifera*.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Retention time</th>
<th>Kovats index</th>
<th>Relative area in percentages</th>
<th>Source of laboratory standards*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uninfected berries</td>
<td>*Botrytis cinerea-*infected berries</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1.33</td>
<td>838.52</td>
<td>-</td>
<td>0.66</td>
</tr>
<tr>
<td>Isobutylaldehyde</td>
<td>1.62</td>
<td>879.47</td>
<td>-</td>
<td>0.03</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.65</td>
<td>882.40</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>2.04</td>
<td>926.03</td>
<td>3.02</td>
<td>1.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.54</td>
<td>970.72</td>
<td>6.01</td>
<td>72.2</td>
</tr>
<tr>
<td>Ethyl propionate</td>
<td>2.72</td>
<td>984.85</td>
<td>-</td>
<td>0.74</td>
</tr>
<tr>
<td>Propyl acetate</td>
<td>2.95</td>
<td>1001.47</td>
<td>-</td>
<td>0.76</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>3.56</td>
<td>1039.60</td>
<td>-</td>
<td>0.58</td>
</tr>
<tr>
<td>1-butanol</td>
<td>6.9</td>
<td>1174.92</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>3-methyl-1-butanol</td>
<td>8.81</td>
<td>1279.64</td>
<td>3.3</td>
<td>8.6</td>
</tr>
<tr>
<td>2-Ethoxyethanol</td>
<td>9.13</td>
<td>1288.55</td>
<td>0.34</td>
<td>-</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>10.5</td>
<td>1324.12</td>
<td>-</td>
<td>0.76</td>
</tr>
<tr>
<td>2-Octanone</td>
<td>10.87</td>
<td>1333.07</td>
<td>1.0</td>
<td>0.58</td>
</tr>
<tr>
<td>Acetoin</td>
<td>11.12</td>
<td>1338.71</td>
<td>3.32</td>
<td>-</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>13.37</td>
<td>1385.58</td>
<td>22.0</td>
<td>2.85</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>14.2</td>
<td>1490.02</td>
<td>-</td>
<td>0.59</td>
</tr>
<tr>
<td>3-Hexen-1-ol (E)</td>
<td>15.05</td>
<td>1508.09</td>
<td>28.0</td>
<td>0.02</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>16.58</td>
<td>1538.18</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>16.77</td>
<td>1541.91</td>
<td>2.2</td>
<td>0.73</td>
</tr>
<tr>
<td>2-ethyl-1-hexanol</td>
<td>18.1</td>
<td>1565.45</td>
<td>7.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>18.3</td>
<td>1568.87</td>
<td>0.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>20.21</td>
<td>1599.84</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>Carbitol</td>
<td>21.48</td>
<td>1078.68</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>28.1</td>
<td>1961.14</td>
<td>0.9</td>
<td>0.08</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>28.44</td>
<td>1966.25</td>
<td>1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>29.31</td>
<td>1979.06</td>
<td>-</td>
<td>2.56</td>
</tr>
</tbody>
</table>

* SA—Sigma-Aldrich; MK—Merck; The remainder have been determined following either NIST or AnalyzerPro databases.
Figure 8.4. Response of female *E. postvittana* in a ‘two-choice’ experiment towards *B. cinerea*-infected berries (■) and uninfected berries (□) of *V. vinifera*. *p*<0.05

Figure 8.5 Mean number of eggs laid by *E. postvittana* in two-choice experiment in response to *B. cinerea*-infected (■) and uninfected (□) berries of *V. vinifera*. Error bars denote SEM. ***p*<0.001
Figure 8.6 Response of female *E. postvittana* in a ‘two-choice’ experiment towards ethanol+berries (■) and berries (□) of *V. vinifera*. *p*<0.05

Figure 8.7 Response of female *E. postvittana* in a ‘two-choice’ experiment towards 3-methyl-1-butanol+berries (■) and control+berries (□) of *V. vinifera*. *p*<0.05
Figure 8.8 Mean number of eggs laid by *E. postvittana* in two-choice experiment in response to ethanol+berries (■) and control+berries (□) berries of *V. vinifera*. Error bars denote SEM. ***p<0.001.

Figure 8.9 Mean number of eggs laid by *E. postvittana* in two-choice experiment in response to 3-methyl-1-butanol+berries (■) and control+berries (□) berries of *V. vinifera*. Error bars denote SEM. ***p<0.001.
Figure 8.10 Response of female *E. postvittana* in a ‘two-choice’ experiment towards 3-methyl-1-butanol (■) and control (□). *p<0.05.

Figure 8.11 Mean number of eggs laid by *E. postvittana* in two-choice experiment in response to 3-methyl-1-butanol (■) and control (□). Error bars denote SEM. *p<0.05.
are available (Magan & Evans, 2000). Ethanol is a common product of fermentation that occurs in decomposing plant tissues as a result of injuries inflicted by various biotic or and abiotic factors (Kelsey & Joseph, 2003; Becher et al., 2012). 

*Botrytis cinerea* is a necrotrophic pathogen and under conducive environmental conditions produces pectolytic and cellulolytic enzymes that macerate infected host tissues. Negligible concentrations of ethanol (6.0%) and 3-methyl-1-butanol (3.3%) were detected in uninfected berries, but the same compounds were detected from *B. cinerea* infected berries at concentrations of 72.2% and 8.6%, respectively. These compounds previously have been implicated in the decay and degeneration of *V. vinifera* tissues caused by *B. cinerea* (Bock et al., 1986; Tasin et al., 2012).

Concentrations of alcohols, particularly those of 3-methyl-1-butanol, isobutanol, and ethanol occurred in elevated levels in *B. cinerea* infected berries of *F. ananassa* (Vandendriessche et al., 2012). Another compound, 2-phenylethanol, generated in *B. cinerea* infected berries is a common volatile released during fungal infections of plants. For example, the fruits of *Malus domestica* (Rosales: Rosaceae) infected with *Metschnikowia andauensis* (Saccharomycetales: Metschnikowiaceae) releases 2-phenylethanol (Witzgall et al., 2012). In this study, 2-phenylethanol manifested itself only in *B. cinerea* infected berries, indicating a possible role in the deterrence of *E. postvittana*. These data conflict with the role the same volatile played in the attraction of *Argyresthia conjugella* (Lepidoptera: Yponomeutidae) when synthetic volatiles were tested under laboratory conditions (Knudsen et al., 2008).

In the present study, the decline in the quantities of 1-hexanol, 3-hexen-1-ol (*E*), benzyl alcohol, and 1-butanol, and the increase in the quantity of ethanol and 3-methyl-1-butanol in *B. cinerea* infected berries indicate their role in deterring *E. postvittana*. High levels of ethyl acetate (0.49%) and acetaldehyde (0.08%) in yeast infected berries along with low levels of ethanol and 3-methyl-1-butanol have been shown to attract *L. botrana* (Tasin et al., 2011). In the present study, negligible levels of ethyl acetate (1.2%) and acetaldehyde (0.66%) in *B. cinerea* infected berries along with high levels of ethanol and 3-methyl-1-butanol repelled *E. postvittana*. 
4.2. Volatiles from B. cinerea infected berries inhibit attraction and oviposition of E. postvittana

In the present study, the B. cinerea infected berries of V. vinifera did not stimulate oviposition significantly in gravid E. postvittana adults. In my earlier work (Rizvi et al., 2015a, b; Rizvi & Raman, 2016b), I have demonstrated that the olfactory cues play a key role in the host-seeking behaviour of E. postvittana. Adults of E. postvittana show varying responses to plant volatiles including alcohols, ketones, aldehydes, esters, and terpenes (Suckling et al., 1996; Jordan et al., 2009). For example, citral inhibits attraction of gravid females and deters oviposition (Suckling et al., 1996). On the other hand, eugenol and geraniol do not affect attraction but inhibit oviposition (Suckling et al., 1996). Among polyphagous insects the quantity of deterrence determines the host range (Bernays & Chapman, 1994). Inhibition of oviposition in E. postvittana by B. cinerea infected berries of V. vinifera could be due to higher levels of alcohols released by degrading plant tissues due to fungal infection. In the present study, I found that the olfactory stimuli from B. cinerea infected berries reduced attraction in E. postvittana, although only 10% of the gravid females of E. postvittana landed on uninfected berries of V. vinifera in the wind-tunnel assay. Foster and Howard (1998) found that selection of a plant by E. postvittana for oviposition is principally mediated by plant stimuli perceived while on the plant, whereas olfactory cues were less effective. Oviposition behaviour in E. postvittana is strongly influenced by tactile stimuli (Foster et al., 1997) and the role of tactile cues from B. cinerea infected V. vinifera has recently been explored (Rizvi et al., 2015a, b).

Both attractants and deterrents mediated oviposition by E. postvittana (Suckling et al., 1996). A compound may attract or deter a plant-feeding insect depending on the release rate of that compound (Smart & Blight, 2000). Many green leaf volatiles attract different Lepidoptera (Reddy & Guerrero, 2001). For instance, 3-Hexen-1-ol (E) and 1-hexanol attracted P. xylostella (Reddy & Guerrero, 2001). In the present study, the low quantity of 3-Hexen-1-ol (E) and 1-hexanol, and the absence of 1-butanol, acetoin, and carbitol from B. cinerea infected berries could make the berries less attractive to E. postvittana. In this study, a 1000 µg dose of ethanol in the presence of uninfected berries of V. vinifera inhibited oviposition,
whereas 10 and 100 µg doses of ethanol did not inhibit oviposition by *E. postvittana* under same conditions. In contrast, doses of 10, 100, and 1000 µg of ethanol in the absence of berries of *V. vinifera* had no effect on oviposition indicating that the concentrations of ethanol and the range of volatiles emanating from uninfected berries of *V. vinifera* influenced oviposition by *E. postvittana*. For example, a high concentration of ethanol from *B. cinerea* infected berries of *V. vinifera* inhibited oviposition in *L. botrana*. On the other hand, a low concentration of ethanol produced by yeast infected berries of *V. vinifera* stimulated oviposition (Tasin *et al*., 2011). A similar behaviour also occurred with *D. melanogaster* (Becher *et al*., 2012). Recognition of 3-methyl-1-butanol, produced by both yeasts and fungi, could also be regulating oviposition (Magan & Evans, 2000; Tasin *et al*., 2012). For example, females of *L. botrana* were attracted to yeast infected berries of *V. vinifera*, which released 3-methyl-1-butanol that constituted 13.2% of the total volatile concentration (Tasin *et al*., 2011). The present study demonstrates that a 10 µg dose of 3-methyl-1-butanol in the absence of berries of *V. vinifera* attracted gravid females of *E. postvittana* to lay significantly more number of eggs. 3-methyl-1-butanol occurs in many plants, such as *M. domestica*, *Actinidia delicosa* (Ericales: Actinidiaceae) (Jordan *et al*., 2002), and *F. ananassa*, which are also known as host plants for *E. postvittana*. Therefore, it is possible that 3-methyl-1-butanol acts as a generic attractant for *E. postvittana*, since it has been shown to play a role in attracting several other species of Noctuidae (Lepidoptera) (Landolt & Alfaro, 2001). In the present study, a 100 µg dose of 3-methyl-1-butanol in the presence of the berries of *V. vinifera* inhibited attraction of and oviposition by *E. postvittana*. When exposed to synthetic lures based on the volatile profile of *V. vinifera* at lower doses (e.g., 150 µg), oviposition by *L. botrana* was stimulated, whereas at higher doses (e.g., 1500 µg) oviposition was inhibited (Anfora *et al*., 2009). Therefore, it is possible that a higher dose of 3-methyl-1-butanol (100 µg) supplemented by the natural volatiles emanating from berries communicate a message deterring the females of *E. postvittana* towards oviposition.
5. CONCLUSION

In the present study, low concentrations of ethanol (10 µg or 100 µg) and 3-methyl-1-butanol (10 µg) in the presence of berries of *V. vinifera* did not deter *E. postvittana* to oviposit. This could be a reason why I found no significant inhibition of oviposition by *E. postvittana* when tested on mildly infected berries. Concentrations of volatiles released by the *B. cinerea* infected berries are several times greater than those released by the uninfected berries (Tasin et al., 2012). Therefore, *B. cinerea* induced volatile signals appear to be detected within the volatile profile of the vegetative background of *V. vinifera*. Host-seeking females thus discriminate and distinguish between infected and uninfected berries of *V. vinifera*, and land at the most suitable site of the host.

6. REFERENCES


Chapter 9

General discussion and conclusion
This page is intentionally left blank
1. GENERAL DISCUSSION

The aim of this thesis is to explore the three-way interaction among *E. postvittana*, *B. cinerea*, and *V. vinifera*, by testing the hypothesis that Chardonnay is the most susceptible variety for *E. postvittana* in Australia and infection of *V. vinifera* by *B. cinerea* influences the oviposition behaviour and life-history performance of *E. postvittana*. In this chapter, key findings in terms of variation in volatile composition of *B. cinerea*-infected leaves and berries of *V. vinifera* and its influence on the oviposition behaviour and life-history performance of *E. postvittana* will be discussed briefly, keeping the overall context of the thesis. The covenant here is that chapters 2–8 include discussions of their own.

1.1. Chardonnay the most susceptible variety to *E. postvittana* in Central West New South Wales, Australia

The quality of food is one vital factor that influences and determines life-history performance of insects (Dixon, 1987). Larval diet plays an important role in determining the larval growth and fecundity of most insects (Svoboda *et al.*, 1994) and the Lepidoptera in particular (Mondy *et al.*, 1998), because the energy necessary for oviposition and egg development is principally derived from the nutrients stored during larval stages. Longer development time, lower pupal mass, higher mortality rate, and greater numbers of instars indicate suboptimal quality of the host plants (Frago & Bauce, 2014).

Life-history traits of the Tortricidae vary with variations in host quality (Danthanarayana *et al.*, 1995, Moreau *et al.*, 2006). Nutritional quality varies between plant species and also among varieties (Moreau *et al.*, 2006). For example, *L. botrana*, when raised on the berries of *V. vinifera* varieties Chardonnay, Chasselas, Gewurztraminer, Grenache, and Lambrusque, their larval-developmental time, fecundity, egg size, and the proportion of larval hatching varied significantly (Moreau *et al.*, 2006). *Epiphyas postvittana* larvae are voraciously polyphagous and can utilize plant materials of different nutritive values. *Epiphyas postvittana* feeds on several plants belonging to more than 121 plant families (Suckling &
Brockerhoff, 2010) including several varieties of *V. vinifera*. Varietal differences in *V. vinifera* influence the life-history performance of *E. postvittana*. The larvae of *E. postvittana* fed on leaves of Chardonnay showed a better life-history performance such as they developed quicker than those reared on leaf materials of other varieties, attained greater pupal mass, and performed well as adults (Chapter 4, Rizvi & Raman, 2015b). On the other hand, feeding on Marsanne resulted in slow larval development and poor adult performance. Female-fitness index of *E. postvittana* showed that the larvae raised on Chardonnay showed the greatest female fitness indicating it to be the most preferred host (Chapter 2, Rizvi & Raman, 2016a).

Successful insect feeding on the host is influenced by several factors. Among them, plant nutritive values and plant secondary chemistry are important (Smiley & Wisdom, 1985). Nitrogen is one key limiting nutritional factor for leaf-feeding Lepidoptera (Awmack & Leather, 2002). For instance, the metabolites necessary for building egg yolk, such as essential amino acids in some plant-feeding insects depend exclusively on nitrogen content in their larval diet (Stockhoff, 1993; O’Brien et al., 2002; Chen et al., 2008). Nitrogen content was the greatest in the leaves of Chardonnay and the least was in those of Marsanne, when compared with those of Sauvignon Blanc, Merlot, and Sémillon (Chapter 2, Rizvi & Raman, 2016a) whereas, other factors such as cations, total carbohydrates, and phenols were not (Chapter 2, Rizvi & Raman, 2016a). Such a variation determines larval and adult performances among the Lepidoptera, which require stored energy for reproductive performance (Thiéry & Moreau, 2005, Moreau et al., 2006). Higher levels of nitrogen in the leaves of Chardonnay can be one factor involved in enabling a better life-history performance of *E. postvittana*, similar to the larval growth of *Heliconius ismenius* and *H. melpomene* (Lepidoptera: Nymphalidae) correlating with the nitrogen level in species of *Passiflora* (Passifloraceae) (Smiley & Wisdom, 1985).

### 1.2. Botrytis cinerea infection influences the bionomics of *E. postvittana*

#### 1.2.1 Botrytis cinerea infection alters the oviposition behaviour of *E. postvittana*

Among plant-feeding insects, chemical cues from plants are vital for host selection. Female insects use sensory cues to locate and even assess food and/or oviposition
sites (several examples in Schoonhoven et al., 2005; Bruce et al., 2010). Fungi induce variations in plant chemistry, which eventually alter the volatile composition and thus influence the insect choices (Cardoza et al., 2003, Raman et al., 2012). Plants infected by fungi emit volatile compounds, such as alcohols, carbonyls, and hydrocarbons (Morath et al., 2012). Among alcohols, ethanol is the most common volatile released during fungal infection of plants (Morath et al., 2012). *Botrytis cinerea*, a necrotrophic fungus, is known to produce pectolytic and cellulolytic enzymes that macerate the infected host tissues and release volatile compounds, such as 3-methyl-1-butanol, which is an indicator of decaying plant tissues (Magan & Evans, 2000; Tasin et al., 2012). *Botrytis cinerea* infection changes the volatile composition of leaves and berries of *V. vinifera*. Infection of *V. vinifera* berries by *B. cinerea* increased the production of ethanol from six (uninfected berries) to 72.2% (infected berries) and 3-methyl-1-butanol from 3.3 (uninfected berries) to 8.6% (infected berries) (Chapter 8, Rizvi & Raman, 2016b). High levels of ethanol (c. 80%) in *Fusarium culmorum*-infected *Triticum aestivum* are known (Börjesson et al., 1989). Ethanol is produced by the fungus during fermentation of carbohydrates, which indicates decay and degeneration in organic tissues. Concentration of alcohols particularly 3-methyl-1-butanol and ethanol has been shown to increase in berries of *Fragaria x ananassa* infected by *B. cinerea* (Vandendriessche et al., 2012). *Botrytis cinerea* infection on *V. vinifera* berries produced 2-phenylethanol (Chapter 8, Rizvi & Raman, 2016b), another common volatile released during fungal infections of plants (Witzgall et al., 2012). 2-phenylethanol was one key compound identified in the headspace of the yeast-fermented sugar baits (El-Sayed et al., 2005). 2-phenylethanol may act as attractant or deterrent for the Lepidoptera. For example, *Trichoplusia ni* (Lepidoptera: Noctuidae) were attracted towards 2-phenylethanol (Heath et al., 1992) whereas, *Autographa californica* (Lepidoptera: Noctuidae) were not (Landolt et al., 2001). Such physiological changes in host plant and the consequent release of volatiles from *B. cinerea*-infected berries could be responsible for the deterrence behaviour in adult males and females of *E. postvittana*. Gravid females of *E. postvittana* do not prefer to oviposit on *B. cinerea*-infected berries (Chapter 7, Rizvi et al., 2015a; Chapter 8, Rizvi & Raman, 2016b). Although, the larvae of *E. postvittana* experienced an improved life-history performance when fed
on *B. cinerea*-infected berries of *V. vinifera*, the gravid females do not prefer to oviposit on infected berries.

I found a significant level of attraction of *E. postvittana* adults towards low level of 3-methyl-1-butanol but the deterrence behaviour of *E. postvittana* in the presence of high level of 3-methyl-1-butanol indicates its potential importance in the field management of *E. postvittana*. 3-methyl-1-butanol could potentially act as a chemical deterrent to *E. postvittana* populations. However, the caveat remains that the further investigations are necessary to confirm its role in this context.

*Botrytis cinerea* infection altered the quality and quantity of volatiles emanating from *V. vinifera* leaves (Chapter 5), which in turn changed the oviposition behaviour of *E. postvittana* (Chapter 3, Rizvi *et al.*, 2015b; Chapter 8, Rizvi & Raman, 2015b). Fungi-induced volatiles influence the oviposition behaviour in plant feeding insects. For example, the infection of *Arachis hypogaea* by *Sclerotium rolfsii* released *E*-4,8–dimethyl–1,3,7–nonatriene, (*E,E*)–4,8,12–trimethyl–1,3,7,11–tridec –atraene, methyl salicylate, and 3–octanone, which, in turn, attracted *Spodoptera exigua* (Cardoza *et al*., 2002). Infection of *V. vinifera* by *B. cinerea* released different lipoxygenase compounds such as hexanal, 1–hexanol, 2–hexen–1–ol (E), 2–hexanal (E), and 1–octen–3–ol (Chapter 5). Volatile compounds from the lipoxygenase pathway were the green-leaf volatiles and these account for >50% of emissions from damaged plants (Holopainen, 2004). In addition to the emission of lipoxygenase compounds, alcohols, ketones, aldehydes, aromatics hydrocarbons, and terpenes among the volatiles arised from *B. cinerea*-infected *V. vinifera* leaves (Chapter 5). The level of infection can play a key role in influencing the preference of the Lepidoptera (Tasin *et al*., 2012; Chapter 3, Rizvi *et al*., 2015b). The test of preference patterns of *E. postvittana* indicates that gravid females avoided *B. cinerea*-infected leaves during oviposition (Chapter 3, Rizvi *et al*., 2015b; Chapter 4, Rizvi & Raman, 2015b) and the rate of oviposition was inversely related to levels of infection. An infected plant could still be a source of food for the Lepidoptera (Cardoza *et al*. 2003a; Hatcher, 1995). However, the time difference between egg laying and hatching (the eggs of *E. postvittana* require 7–8 days to hatch at 21 °C) could increase the level of infection and therefore, the potential larval food could deteriorate in quality (Tasin *et al*., 2012). In such
contexts, the avoidance of moderately (30–60%) and intensely (90–100%) \textit{B. cinerea}-infected sites on \textit{V. vinifera} leaves by the ovipositing \textit{E. postvittana} suggests that this could be an adaptive strategy of \textit{E. postvittana} ensuring suitable feeding sites for their offspring (Chapter 3, Rizvi \textit{et al}., 2015b).

Oviposition behaviour in \textit{E. postvittana} is strongly influenced by tactile cues (Foster \textit{et al}., 1997). The number of eggs laid on Dixie cup walls in the ambience of volatiles arising from \textit{B. cinerea}-infected leaves of \textit{V. vinifera} in ‘effect of volatile’ assay was greater than the number of eggs laid on \textit{B. cinerea}-infected leaves in ‘no-choice’ assay (Chapter 3, Rizvi \textit{et al}., 2015b). This indicates that the tactile cues play an important role in oviposition by \textit{E. postvittana}. Gravid \textit{E. postvittana} prefers to oviposit on smooth surfaces with varicose texture rather than on rough and hairy surfaces. Adults of \textit{E. postvittana} lay more eggs on the adaxial leaf surfaces, which bear fewer hairs than the abaxial surfaces (Tomkins \textit{et al}., 1991). Growth of \textit{B. cinerea} on berries and/or leaves of \textit{V. vinifera} results in a physical and enzymatic breakdown of the epidermal cell walls. Growing hyphae spread along the epidermal and subepidermal cell layers, emerge close to the surface, burst through the cuticle, and form conidiophores on berry skin (Magya & Bene. 2006). The mycelial bed of \textit{B. cinerea}, which is apparently similar to trichomatous leaf surfaces possibly, influences the ovipositional behaviour of \textit{E. postvittana} (Chapter 3, Rizvi \textit{et al}., 2015b).

1.2.2. \textit{Botrytis cinerea} alters the behaviour of \textit{E. postvittana} larvae

The larvae of \textit{E. postvittana} carry viable conidia of \textit{B. cinerea} on their body surfaces and in gut (Chapter 3, Rizvi \textit{et al}., 2015b) and can transmit them from infected tissues to uninfected tissues of \textit{V. vinifera} (Chapter 7, Rizvi \textit{et al}., 2015a). The larvae of \textit{E. postvittana} showed preference for the mildly (5–10%) and moderately (30–60%) infected leaves of \textit{V. vinifera} but showed no significant preference for the intensely (90–100%) infected leaves of \textit{V. vinifera} (Chapter 3, Rizvi \textit{et al}., 2015b). The larvae of \textit{E. postvittana} did not complete their life cycles on intensely (90–100%) infected leaves of \textit{V. vinifera} and died before pupation, whereas feeding on mildly (10–30%) infected leaves improved their life-history performance (Chapter 3,
Rizvi & Raman, 2015b). In such a context, no preference for the intensely (90–100%) infected leaves of *V. vinifera* impresses as an adaptive strategy of *E. postvittana* larvae ensuring their survival. *Botrytis cinerea* infection of berries of *V. vinifera* did not significantly inhibit the attraction of *E. postvittana* larvae (Chapter 7, Rizvi *et al.*, 2015a). In response to *B. cinerea* infection, *V. vinifera* leaves accumulate defence compounds including several possible anti-insectan compounds, such as chitinase and β-1,3-glucanase (Busam *et al.*, 1997; Derckel *et al.*, 1999; Vihervuori *et al.*, 2013). Accumulation of these compounds in leaves in response to the *B. cinerea* infection could be one reason for the mortality of the larvae of *E. postvittana*, while rearing them on *B. cinerea*-infected leaves of *V. vinifera* (Chapter 5). Whereas, with the onset of ripening (*véraison*) of berries, some of the defence compounds either do not manifest or possibly they get modified into non-defensive compounds (Jeandet *et al.*, 1995). On the other hand, fungal infection can decompose complex organic material into simpler forms (Cardoza *et al.*, 2003a).

These could be the reasons why the larvae of *E. postvittana* experienced a better life-history performance while feeding on *B. cinerea*-infected berries (Chapter 7, Rizvi *et al.*, 2015a). In polyphagous insects, such as *E. postvittana*, the quality and quantity of deterrent compounds in plants determine the host-plant range (Bernays & Chapman, 1994; Suckling & Brockerhoff, 2010). Visual cues play a role in orientating larvae (Harris *et al.*, 1995). Upon hatching, the larvae of *E. postvittana* did not distinguish between potential host and non-host from a distance (Harris *et al.*, 1995). For example, the larvae of *E. postvittana* did not show an initial preference between the diet enriched with *B. thuringiensis* δ-endotoxins (cryIAc and cryIBa) or control diet (diet without *B. thuringiensis* δ-endotoxins) and over time the larvae rejected δ-endotoxin-included diet and gathered on control diet (Harris *et al.*, 1997) suggesting that gustatory cues influence their decision enabling them to either progress with the feeding or not (Suckling & Ioriatti, 1996, Harris *et al.*, 1999). The larvae of *E. postvittana* showed no significant preference for artificial diet incorporated with lyophilized mycelium of *B. cinerea* over the artificial diet without the fungus material (Chapter 6, Rizvi & Raman, 2015a), although the artificial diet incorporated with lyophilized mycelium improved life-history performance of larvae. Both diets (artificial diet incorporating lyophilized mycelium and the control) possibly did not include either a deterrent or an anti-nutritional compound, which
may have influenced the feeding preference of *E. postvittana* larvae; and this seems a more likely explanation for the significant preference for artificial diet incorporated with lyophilized mycelium of *B. cinerea* or control.

1.2.3. *Botrytis cinerea* infection alters *E. postvittana* life-history

Insect and fungus association ranges from non-specific polyphagy to obligate mutualism (Jonsell & Nordlander, 2004). The level of infection can influence the mutualistic relationship between insect and fungus (Biere & Tack, 2013). For example, low level of infection of *Pinus radiata* (Pinaceae) by *Ophiostoma ips* (Ophiostomatales: Ophiostomataceae) enabled adult *Ips pini* (Coleoptera: Scolytinae) in settling and colonizing by drying the sapwood of *P. radiata*, but the intense level of infection had a detrimental effect on the development of *I. pini* larvae (Kopper et al., 2004). *Epiphyas postvittana* larvae showed better life-history performance when fed on mildly (10–30%) *B. cinerea*-infected leaves (Chapter 4, Rizvi & Raman, 2015b) but they experienced total mortality when fed on >70% *B. cinerea* infected leaves of *V. vinifera* (Chapter 4, Rizvi & Raman, 2015b; Chapter 5). Fungal infection can improve insect nutrition either by breaking the complex materials into simpler forms or by decreasing the level of defence compounds (Cardoza et al., 2003a; Hatcher, 1995). For example, leaves of *A. hypogaea* infected by *S. rolfsii* showed significantly higher soluble sugar but significantly lowered soluble phenolics compared with healthy leaves (Cardoza et al., 2003a). However, the infection may deteriorate the potential food and may increase chances of mycotoxicity (Clay, 1987; Tasin et al., 2011). Fungal infection can also decrease the nitrogen levels in the host tissue (Hatcher, 1995). The ratio between carbohydrate and nitrogen play a key role in insect development (Clancy, 1992). The larvae of *Choristoneura occidentalis* (Lepidoptera: Tortricidae) experienced deleterious growth when fed on high carbohydrate and low nitrogen food (Clancy, 1992). The larvae of *Gastrophysa viridula* (Coleoptera: Chrysomelidae) experienced longer developmental time and higher larval mortality when fed on *Uromyces rumicis* (Pucciniales: Pucciniaceae)-infected *Rumex* species (Polygonaceae) than that fed on uninfected *Rumex* leaves (Hatcher et al., 1994). This detrimental effect on
The performance of *G. viridula* was mainly associated with low nitrogen content of *U. rumicis*-infected *Rumex* leaves (Hatcher et al., 1995). *Botrytis cinerea* displays the capacity to kill host cells by producing mycotoxins such as botrydial, botrydienal, and 8,9-epibotrydial (Hof & Kupfahl, 2009). Insects differ in their ability to destroy mycotoxins, for example, the larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) was able to detoxify 4-monoacetoxyscirpenol (mycotoxin produced by many crop infecting fungi including *Fusarium* species) more effectively than that of *Helicoverpa zea* (Lepidoptera: Noctuidae) (Dowd & Van Middlesworth, 1989; Dowd, 1989). Moreover, sometimes mycotoxin can synergize the toxicity of plant allelochemicals (Hatcher, 1995). For example, fusaric acid produced by *Fusarium* species synergized with the allelochemical of *Gossypium hirsutum* (Malvaceae) and increased the mortality and decreased the development rate of larvae of *H. zea* (Dowd, 1989). Cardoza et al. (2003a) used a sublethal infection of *S. rolfsii* to raise the larvae of *S. exigua* and concluded that high intensity of fungal population reverses the positive effect on the larvae.

Many arthropods have specialized consuming fungal mycelial and spores (Hatcher, 1995). Fungal tissues contain water, carbohydrates, proteins, B vitamins, choline, and sterols (Hatcher, 1995; Morales-Ramos et al., 2000). *Tetranychus urticae* (Trombidiformes: Tetranychidae) prefer to lay eggs on *Uromyces appendiculatus* (Uredinales: Pucciniaceae)-infected *Phaseolus vulgaris* and the larvae intimately feeds on *U. appendiculatus* (Batra & Stavely, 1994). Laemmilen and Hall (1973) found that larvae of *Siteroptes reniformis* (Acari: Siteroptidae) only established on *Nigrospora oryzae* (Trichosphaeriales: Trichosphaeriacaeae)-infected *G. hirsutum* and could not live on uninfected *G. hirsutum*. Insects in general and Lepidoptera in particular lack the capacity to synthesize essential sterols to build key hormones and other lipid molecules that moderate development. Plant-feeding insects acquire sterols (or sterol precursors) from plants and/or from the microbial symbionts (Mondy & Corio-Costet, 2000, Behmer & Nes, 2003). In several insect–fungus symbioses, the insect depends on sterols provided by the fungus (Behmer & Nes, 2003). The Scolytinae are dependent on ergosterol produced by their fungal symbionts for successful oocyte development, oviposition, larval development, and pupation (Bentz & Six, 2006). *Hypothenemus hampei* fed on the berries of *C.*
*arabica*, but could not either moult or reproduce without ergosterol from *F. solani* that occurs on *C. arabica* (Morales-Ramos et al., 2000). Although, phytosterols are easily transformed by phytophagous insects into cholesterol by clemethylation, providing them with a source of sterol for the biosynthesis of cholesterol. This is less obvious for the ergosterol, which is a poly-unsaturated sterol, to metabolize into cholesterol (except for specific insects with symbiotic relationship, such as scolytinae). The evolution of phytosterol contents of *V. vinifera* berries showed that with maturation, phytosterol values in berry skins dropped significantly (Le Fur et al., 2015), which possibly rendered the berries less preferable to the *E. postvittana* larvae. In this context, the association of *E. postvittana* with *B. cinerea* may supply the necessary sterols and vitamins (Mondy & Corio-Costet, 2000) to the developing larvae of *E. postvittana*. Infection by *B. cinerea* breaks complex sugars into simpler forms (Tasin et al., 2011, 2012) and involved in decreasing the levels of defence compounds in berry skin (Cardoza et al., 2003a). Moreover, with *véraison* of berries, some of the defence compounds either do not manifest or modifies into non-defensive compounds (Jeandet et al., 1995). This seems to be the most appropriate explanation why larvae of *E. postvittana* show a better life-history performance when fed on *B. cinerea*-infected berries compared to that fed on uninfected berries of *V. vinifera* (Chapter 7, Rizvi et al. 2015a). On the other hand, the production of defence compounds including several possible anti-insectan compounds due to the growing *B. cinerea* infection can make the *V. vinifera* leaves toxic to the developing larvae and therefore, could well be responsible for the total mortality of larvae fed on leaves infected <70% by *B. cinerea* (Chapter 5). Fungal infection often increases the dry matter in infected tissues due to increase in fungal mass, or, possibly, changes in plant cellular structure (Farrar & Lewis, 1987). Infection by *Drechslera siccans* (Pleosporales: Pleosporaceae) significantly reduced *in vitro* dry matter digestibility, water-soluble carbohydrate and the total amino acid content of *Lolium multiflorum* (Poaceae) foliage (Lam, 1985). Lower concentrations of digestive material reduce the concentration of nutrition. Insects may compensate this by increasing their consumption rate (Hatcher, 1995). During higher consumption, larvae may also consume higher concentration of allelochemicals or mycotoxins, which increases mortality and reduces their development rate (Slansky & Wheeler, 1992).
Some insects feed on fungi either without demonstrating any specialized relationship such as mutualism or may feed on a specific fungus, their relationship ranging from feeding on primary metabolites to secondary metabolites. The larvae of Sirex noctilio (Hymenoptera: Siricidae) have an obligate symbiotic relationship with Amylostereum areolatum (Russulales: Amylostereaceae) and could only survive in A. areolatum-infected Pinus radiata (Pinales: Pinaceae) (Slippers et al., 2003; Yousuf et al., 2014). The larvae of E. postvittana showed a better life-history performance when fed on synthetic diet incorporated with the mycelia material of B. cinerea (Chapter 6, Rizvi & Raman, 2015a). A similar result has been shown in the larval population of L. botrana reared on purified sterols from B. cinerea (Mondy & Corio-Costet, 2000). Reproduction in several plant-feeding taxa has been demonstrated to be closely linked to feeding preferences during larval stages (Awmack & Leather, 2002). For instance, oocyte production depends on the quality of food consumed during larval periods; bulk of nitrogen and carbon components of eggs were derived from the nutrients obtained during larval period (Boggs, 1997). Diet incorporated with B. cinerea significantly enhanced fecundity and fertility of E. postvittana (Chapter 6, Rizvi & Raman, 2015a). Since adult females of E. postvittana fed only on water, all nutrients for egg production and development are derived from the larval feeding (Chapter 6, Rizvi & Raman, 2015a). Similar results have been shown when the larvae of L. botrana were fed on artificial diet incorporated with mycelium of B. cinerea (Tasin et al., 2011) or purified sterols from B. cinerea (Mondy & Corio-Costet, 2000). Aphis fabae (Hemiptera: Aphididae) developed faster, attained heavier pupal mass, and laid greater number of eggs when fed on Botrytis fabae (Helotiales: Sclerotiniaceae)-infected Vicia faba (Fabaceae) (Zebitz & Kehlenbeck, 1991). Similarly, when the larvae of Apion onopordi (Coleoptera: Apionidae) developed on Puccinia punctiformis (Pucciniales: Pucciniaceae)-infected tissues of Cirsium arvense (Asteraceae) laid almost twice as many eggs that fed on uninfected tissues.

1.3. Three-way interacting system

Plants are associated with a variety of microbes and plant-feeding insects, establishing three-way interacting systems. Such interactions can have either
beneficial or detrimental effect on the participants’ performances. Pathogenic fungi, besides altering the metabolism of host plants, can have both positive and negative effects on the population dynamics and life-history performance of associated insects (Stout et al., 2006; Biere & Tack, 2013). For example, *Ostrinia nubilalis* (Lepidoptera: Crambidae) larvae developed approximately 20% faster on *Zea mays* (Poaceae) tissues infected by *Glomerella graminicola* (Glomerellales: Glomerellaceae) than those fed on uninfected tissues of *Z. mays* (Carruthers et al., 1986). *Apion onopordi* showed higher survival rate and better life-history performance, when fed on *P. punctiformis*-infected shoots of *C. arvense* than those fed on uninfected shoots of *C. arvense* (Bacher et al., 2002). On the other hand, Infection of *C. arvense* with the necrotrophic fungus *Phoma destructiva* (Pleosporales: Incertae sedis) reduced oviposition, feeding, survival and growth rates, and pupal mass of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) (Kruess, 2002). Larvae of *Melitaea cynthia* (Lepidoptera: Nymphalidae) experienced reduced growth on *Plantago lanceolata* (Plantaginaceae) infected by *Podosphaera plantaginis* (Erysiphales: Erysiphaceae) (Laine, 2004). In the interacting system of *E. postvittana–V. vinifera–B. cinerea*, life-history performance of *E. postvittana* was significantly influenced when fed on *B. cinerea*-infected berries of *V. vinifera*.

A poor linkage between oviposition preferences by adults and performance of the offspring of *E. postvittana* has been shown (Foster & Howard, 1999). In a choice context of 26 plant species, *E. postvittana* females showed either little or no oviposition preference towards the plant species that have been classified as either hosts or non-hosts for larval performance (Foster & Howard, 1999). Avoidance of a ‘diseased’ plant could be an adaptive strategy among plant-feeding insects. Although infected plants, sometimes, could provide readily available food (by breaking complex food into simpler form) to the developing larvae but intensifying infection may deteriorate the potential food. Gravid *E. postvittana* does not lay eggs on infected site of either leaves or berries, although eggs have been found to occur close to infected sites, particularly in no-choice experiments (Chapter 3, 7, Rizvi et al., 2015a, b). The mycelial bed of *B. cinerea*, which resembles to trichotomous structure, can act like inhibitory tactile cues for *E. postvittana*. 
The larvae of *E. postvittana* are leaf rollers and can complete their life-cycle solely feeding on leaves of *V. vinifera* (Chapter 4, Rizvi & Raman, 2015b). But *E. postvittana* experienced detrimental effect on larval development and reproductive potential when fed on berries compared with those fed on *B. cinerea*-infected berries of *V. vinifera* (Chapter 7, Rizvi et al., 2015a). Lack of sterols in the berries could be one reason for that detrimental effect on *E. postvittana*. *Botrytis cinerea* infection on berries possibly supplements the ‘necessary’ sterols to the developing larvae of *E. postvittana*. On the other hand, the conidia of *B. cinerea* can contaminate leaves or inflorescences, but the fungus develops mainly on the ripe berries of *V. vinifera* (Choquer et al., 2007). *Botrytis cinerea*-infected berries significantly improved larval and adult performance of *E. postvittana* (Chapter 7, Rizvi et al., 2015a) and apparently provided necessary nutrients to the larvae. Furthermore, the larvae of *E. postvittana* carried viable conidia and acted as a vector for conidial dispersal. In such a context, the interaction between *E. postvittana*, and *B. cinerea* in with *V. vinifera*, appears to be mutualistic.

In this study, I hypothesized that Chardonnay is the most susceptible variety to *E. postvittana* infestation and *B. cinerea* infection of *V. vinifera* influences the bionomics and life-history performance of *E. postvittana*. My experiments using artificial diet incorporated with Chardonnay leaf material have shown that such a combination improves the life-history performance of *E. postvittana* compared with the leaf materials of other four tested varieties of *V. vinifera*. The infection of *V. vinifera* by *B. cinerea* changes the volatile profile of leaves and berries of *V. vinifera*, which alter the oviposition behaviour of *E. postvittana*. The infection also significantly influences the life-history performance of *E. postvittana*.

2. **CONCLUSION**

In Australian vineyards, *E. postvittana* adults frequently encounter *B. cinerea* during oviposition, and *E. postvittana* larvae co-occur with *B. cinerea* feeding on their hyphae and spores, establishing a three-way interacting system. The infection level of *V. vinifera* by *B. cinerea* plays a key role in the oviposition behaviour and life-history performance of *E. postvittana*. Olfactory cues along with tactile cues
contribute a major role in the oviposition behaviour of *E. postvittana*. The occurrence of *B. cinerea* and the larvae of *E. postvittana* simultaneously on *V. vinifera* improves the life-history performance of *E. postvittana* indicating a mutualistic relationship among them. Although, *B. cinerea* infection influenced the key life-history parameters of *E. postvittana*, the present study does not support that the fungus involvement is critical for the survival and development of *E. postvittana*.

This study represents an intense study of plant–insect interactions by considering not only the direct effects of the plant (*V. vinifera*) but also the effects of plant–fungus interactions (*V. vinifera–B. cinerea*) on the population dynamics of pestiferous Lepidoptera (*E. postvittana*). This study addresses the susceptibility level of five varieties of *V. vinifera* towards *E. postvittana* infestations. The poor response of *E. postvittana* on the variety Marsanne could be useful in IPM via push—pull strategy by planting Marsanne either near or among the susceptible varieties of *V. vinifera*. Moreover, the deterrence behaviour of *E. postvittana* in the presence of high levels of 3-methyl-1-butanol indicates its usefulness in the management of *E. postvittana*.

3. REFERENCES


Bentz, B.J. and Six, D.L. (2006) Ergosterol content of fungi associated with *Dendroctonus ponderosae* and *Dendroctonus rufipennis* (Coleoptera:


